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CYTOGENETIC STUDIES ON RUMEX SUBGENUS ACETOSELLA

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I. INTRODUCTION.

INVESTIGATIONS at length on the problem of speciation have been undertaken by the taxonomists of the last two or three centuries. The palaeontologists and natural philosophers of the 19th and 20th centuries added to it a discussion at breadth. The depth of the problem is being studied by the modern cytogeneticists and biogeographers, although hitherto their efforts have not been very successful in many groups except *Drosophila*, *Triticum*, *Datura* and some other genera of plants and animals. The time has probably not yet come for an exhaustive treatment of the evolution of species on the basis of investigations of the cytogenetics, geography and taxonomy of whole groups of plants, but some studies on the polymorphic genera in the plant kingdom may elucidate some of the most interesting aspects of the problem. This paper — together with another on the taxonomy and phytogeography of *R.* sg. *Acetosella* (LÖVE, unpubl.) — may be considered a link in the works on the significance of polyploidy in the evolution and speciation in the plant kingdom.

The genus *Rumex* must be regarded as an ideal material for an attack on the cytogenetics, taxonomy and speciation of plants in order to prepare the ground for a joint analysis of the evolutionary processes. It includes about 250 species, which are distributed all over the world. These species are to be divided into three subgenera with well-defined morphological as well as geographical and cytogenetic characteristics.

The largest subgenus is *Lapathum* (CAMPD.) RECH. F., which includes about 150 hermaphroditic species. According to the most modern taxonomical revision of this group (RECHINGER, 1937), it is split up into three sections on a merely morphological basis: *Simplices* RECH. F., *Axillares* RECH. F. and *Platypodium* WILLD. Cytologically the first-mentioned section is characterized by the basic chromosome number $x = 10$, the two others having the basic number $x = 8$ in the material so far investigated. The subgenus is also recognized by the high fre-

quency of polyploidy and by the relatively small chromosomes. The single species of the section *Platypodium*, *R. bucephalophorus* L., seems to form a link between this subgenus and the subgenus *Acetosella*, according to some of its morphological characteristics and the number and morphology of the chromosomes.

The second subgenus, *Acetosa* (CAMPD.) RECH. F., comprises about one hundred different species, belonging to four morphologically and cytologically well-defined sections. The section *Euacetosae* LÖVE includes only dioecious species with the basic number $x = 7$ (8). Its chromosomes are relatively large and all the species have the same diploid number of chromosomes and the same cytogenetic sex mechanism: two *X*-chromosomes and twelve autosomes ($2n = 14$) in the females and one *X*, two *Y*'s and twelve autosomes ($2n = 15$) in the males. The species of *Euacetosae* are perennials. The section *Hastati* LÖVE also embraces only perennial plants, but they are all gynodioecious (or polygamodioecious?) and have the basic number $x = 9$. Within this section no heteromorphous sex chromosomes are detectable at meiosis (ONO, 1940 b; LÖVE, 1943), and only one case of polyploidy has been observed as yet, i. e. in the species *Rumex Lunaria* L. (LÖVE, 1943). The chromosomes of section *Hastati* are somewhat smaller than those of *Euacetosae*. The third section, *Scutati* LÖVE, comprises hermaphroditic perennials with the basic number $x = 10$. The chromosomes are of about the same size as those of section *Hastati*, and one case of polyploidy is also met with in the material of the collective species *R. scutatus* from England (FIKRY, 1930). The last section, *Vesicarii* LÖVE, includes small, annual species from the Mediterranean deserts. Its basic number is $x = 9$, and no case of polyploidy has been met with in the material of the closely related species studied hitherto. The chromosomes are at least as large as those of the section *Euacetosae*.

The subgenus *Acetosella* (MEISN.) RECH. F. is the smallest group of the genus. It consists of only four dioecious perennial species with the basic number $x = 7$ and the diploid, tetraploid, hexaploid and octoploid chromosome numbers respectively. This paper presents the cytogenetic results of an attempt to revise these four species from the standpoints of cytology and genetics and with the multiple needs of taxonomy, phytogeography and physiology borne in mind. The taxonomical and phytogeographical results of the investigation will be published in another paper (LÖVE, unpubl.).

Rumex sg. *Acetosella* is of particular interest in more than one

respect. *Firstly*, its taxonomy has been almost chaotically confused, as may be seen in, for instance, the work by ASCHERSON and GRAEBNER (1908—1913), where it fills up five pages with descriptions of various systematically worthless types differing in highly disputable characteristics. Most taxonomical works, however, fail to distinguish between the different species and types, treating them all merely as »*Rumex Acetosella*». *Secondly*, the behaviour of its sex mechanism in relation to polyploidy may be of fundamental interest in helping us to understand the evolution of this subgenus in particular and the differentiation of sex chromosomes in general. According to ONO (1935) and YAMAMOTO (1938), the sex determination of the dioecious species of subg. *Acetosa* is based on a balance between the female-determining *X*-chromosomes and the mainly male-determining autosomes. This mechanism prevents polyploidy. As the polyploid species of subg. *Acetosella* are dioecious, its sex determination must be explained in some other way than by the mechanism met with in the related species of subg. *Acetosa*. As will be shown later, the evolution of this mechanism has been fundamentally different in the dioecious *Rumex* groups since they became differentiated from their putative common ancestors. *Thirdly*, the investigations into the differences as between species in the two dioecious groups of the genus have shown that the evolution of species within these different groups of the same genus may be guided by different agents. In the subgenus *Acetosa* the speciation seems to be a gradual process based mainly on genic and structural-chromosomal differentiation in geographically isolated colonies. In the subgenus *Acetosella* the polyploidy alone seems to catapult a new species into being. This difference may be due to more than a mere coincidence. *Finally*, examinations of some physiological and other characters in relation to the polyploidy and geographical distribution of this polyploid series, as well as a closer analysis of the cytology of the different species may be of great help in understanding the real differences between the different species.

II. THE DISTRIBUTION OF RUMEX SG. ACETOSELLA.

The results of the investigations into the taxonomy and geography of the different forms of the *Acetosella* group will — as mentioned above — be published in another paper (LÖVE, unpubl.). As, however, it may be of interest to give some account of the gross geographical

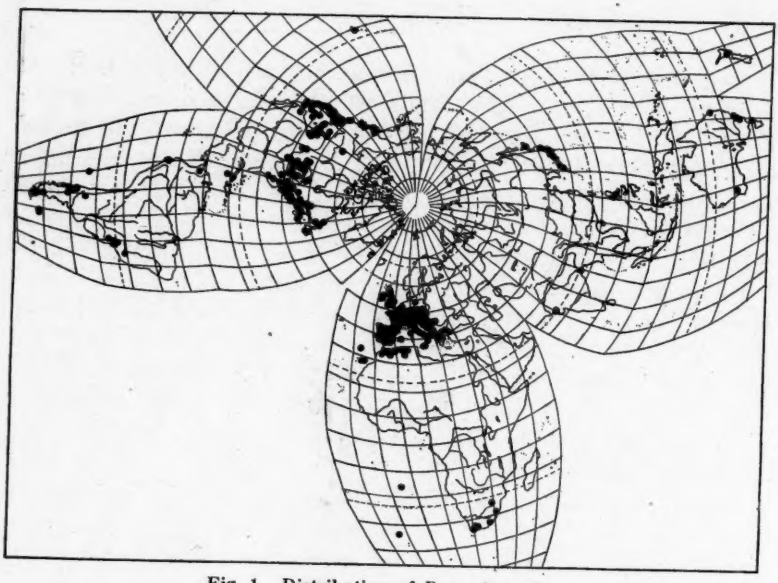


Fig. 1. Distribution of *R. angiocarpus.*

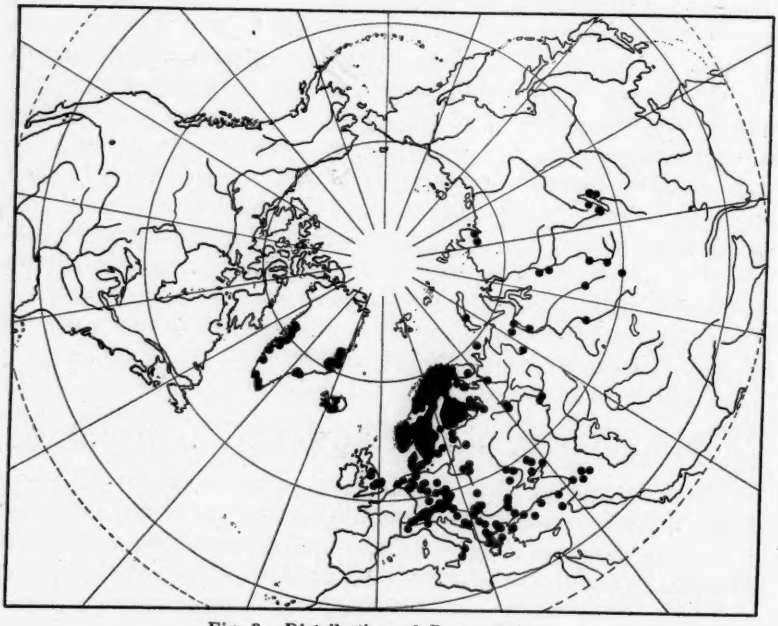


Fig. 2. Distribution of *R. tenuifolius.*

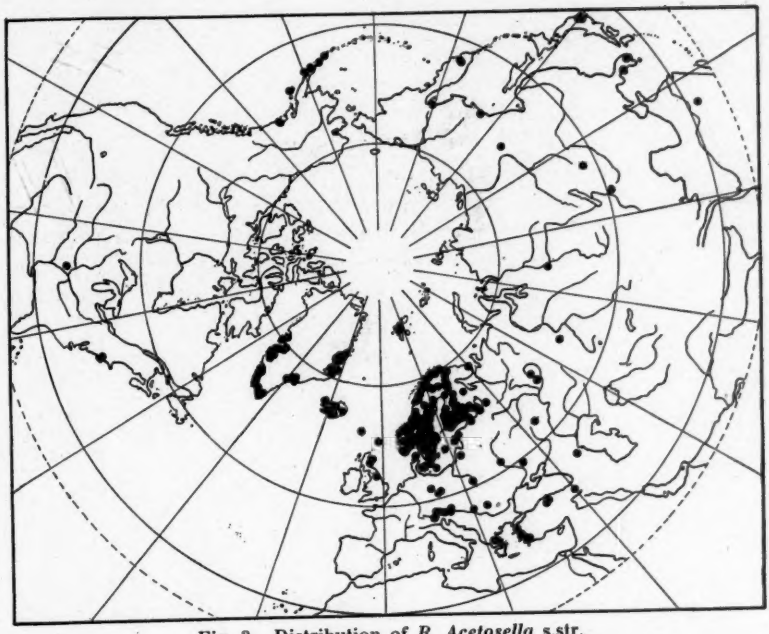


Fig. 3. Distribution of *R. Acetosella* s.str.

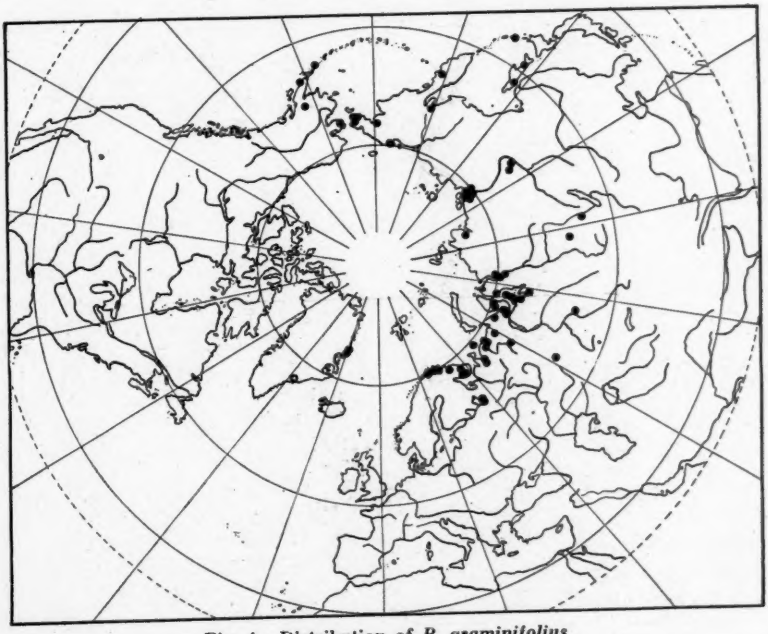


Fig. 4. Distribution of *R. graminifolius*.

distribution of the different species in connection with the cytogenetical results, the following data may be given here.

The species of *Rumex* sg. *Acetosella* are four in number. The taxonomically oldest name is that of *Rumex Acetosella* L., s. str., published by LINNAEUS in 1753. The second species is *Rumex graminifolius* (LAMBERT, 1811), the third species is *Rumex angiocarpus* (MURBECK, 1891), and the taxonomically youngest one is *Rumex tenuifolius* (WALLR.) LÖVE (LÖVE, 1941 a). These four species have been found to form a polyploid series (cf. LÖVE, 1941 b), and their most significant taxonomical characteristics are the size (and shape) of the floral parts and some habitual marks, such as, e. g., the form of the leaves, the mode of growth, etc. It seems that the majority of the characteristics of these four species are caused directly by the differences in chromosome number.

The geographical distribution of the four species has been studied mainly from herbarium material in the Botanical Museums in Scandinavia. All the details of these studies will be given in a later paper, but as the gross distribution is of some interest in connection with the discussion on the age, etc. of the polyploid species of the subgenus *Acetosella* in particular, and the distribution of polyploid plants in general, the maps in Figs. 1—4 have been reproduced.

As will be seen from the maps, *R. angiocarpus* is almost the only species of *Acetosella* in W. and S.W. Europe as well as in America, Africa, Australia and E. Asia (Japan). The species *R. tenuifolius* is only found in the northern hemisphere in Europe and Asia, and it is most common in northern Europe and Siberia. In alpine regions in Scandinavia this is the native species, and it is found in arctic regions in Siberia and Nova Zembla. It is met with in E. Asia, but not in America, except Greenland. *R. Acetosella* s.str. is also found in N. and E. Europe and Asia and in N.W. Alaska. In Siberia it is more southern than the last-mentioned species. In Scandinavia it is met with as an introduced form in cultivated places, at road sides, etc. and in a native form in some parts of the northern regions. The same form is also met with in Iceland and Greenland. It is named ssp. *islandicus* LÖVE. *R. graminifolius* is found almost solely in arctic regions. According to previous research-workers it is only found in Alaska, Siberia, North Russia, Kola, Sachalin, and the Kuriles. The present writer has, however, detected material of different forms of the species from Karelia onegensis, North Norway and N.E. Greenland, as may be seen on the map.

These four maps show the total distribution of the subgenus *Acetosella* as known so far by the present writer. The distribution coincides with the theories on the differences in distribution of diploids and polyploids (cf. LÖVE and LÖVE, 1943) as the diploid species *R.*

angiocarpus is found in more temperate climates, and the octoploid species *R. graminifolius* is the most northern form of the group.

III. MATERIAL AND METHODS.

1. THE MATERIAL.

A. *Rumex angiocarpus.* — The material of this species was obtained from seeds originating from Lisbon, Portugal. The seeds were obtained partly from Professor PALHINHA through the kind assistance of Dr. HELGI P. BRIEM, Lisbon, and partly from the seed-exchange of the Bergian Botanical Gardens in Stockholm. No detailed information as to the locality, etc. of the mother individuals in Portugal were given by the collectors of the seeds. Owing to the present war the writer has not been able to obtain another material of this species.

The material cultivated at Svalöf was composed of 57 flowering individuals and 18 non-flowering plants. The average height of the plants was $49{,}7 \pm 1{,}8$ cm. The males were somewhat shorter than the females, the height being $47{,}6 \pm 5{,}9$ and $51{,}5 \pm 2{,}4$ cm. respectively, but as the material is scanty these differences are not found to be of much significance statistically.

Fig. 5. Distribution in Scandinavia of *R. tenuifolius* and *R. Acetosella* s.str. cytologically studied.

B. *Rumex tenuifolius.* — The material of this species can be divided into three classes: *Firstly*, it originates from seeds from Liège, Belgium; the neighbourhood of Budapest, Hungary; Moscow, USSR.; Murmansk, USSR. and Helsingfors, Finland. These seeds were obtained under the name of *R. Acetosella* from different botanists and Botanical Gardens by the writer himself and by the Botanical Garden at Lund. *Secondly*, a large number of individuals has been collected by the writer himself as well as by several botanists in Sweden, Norway, Finland and Iceland, and cultivated in the experimental fields at Svalöf. Altogether about 90 individuals from about 30 different places in almost all parts of Sweden and some places in the other Nordic countries have so far been cytologically studied (cf. Fig. 5). Perhaps, it would have been of special interest to some groups of botanists to obtain detailed accounts of the origin of every plant (cf. ARWIDSSON, 1938), but as the space is limited no list will be given in this paper. *Thirdly*, a number of hybrids between plants from different localities have been produced and studied.

The average height of the material of *R. tenuifolius* was 41,9 ± 1,6 cm. The males were somewhat shorter than the females, the height being 41,1 ± 2,2 and 43,1 ± 2,1 cm. respectively. These numbers, however, are not statistically different.

C. *Rumex Acetosella s.str.* — Of this species only material from Sweden, Norway, Finland and Iceland has been studied. Chromosome counts have been made in respect of 140 individuals from 33 different localities (cf. Fig. 5) and of a large number of experimentally produced hybrids between plants from different localities. The material from Scandinavia belongs to the main form, but the material from Iceland is composed of individuals of the ssp. *islandicus* (one collection) and of plants of the main form, which is to be found introduced at some places in Iceland.

The average height of the material of *R. Acetosella* s.str. was 44,9 ± 1,2 cm. The males of this species were likewise somewhat shorter than the females, the height being 43,5 ± 1,7 and 45,0 ± 1,8 cm. respectively.

D. *Rumex graminifolius.* — The material of this arctic species originates from the shore of Petchora, USSR. It was collected by Professor A. TOLMATCHEW of Archangelsk and sent to me as seeds in the autumn of 1940. Only a few of them germinated and they were all cultivated in pots. Unfortunately, all the plants died before flowering and therefore only their somatic chromosomes could be examined.

2. CYTOLOGICAL METHODS.

The chromosome number of all the plants cultivated has been determined in the root-tip cells. The root-tips are fixed in the fixative 8 : 2 of LEWITSKY (1931 a): 8 parts of 1 % chromic acid : 2 parts of 10 % formalin (diluted from 40 % formalin), mixed immediately before use. The root-tips are sectioned 13 micra thick and stained according to the iodinekalium-gentian-violet method (cf. NEWTON and DARLINGTON, 1929). Before staining, the objects fixed in LEWITSKY's 8 : 2 must be placed in a mordant solution of 1 % chromic acid during 12 to 24 hours.

The meiotic divisions of all male plants were scrutinized, partly in material fixed in the modification of NAVASHIN's fluid used at Svalöf and Lund, after a short pre-treatment in abs. alcohol or CARNOY's 3 : 1, but mainly permanent smear-preparations were used. These were made according to a modification of the method given by ÖSTERGREN (1942). The flowers were fixed in CARNOY's 3 : 1 and after about 24 hours they were removed to BELLING's iron-acetocarmine with an extra addition of iron. In this mixture the flowers are preserved for at least a week. When smeared, the flowers are boiled in a drop of 45 % acetic acid, and afterwards the procedure is the same as that given by ÖSTERGREN (l. c.). The preparations are made permanent in Canada-balsam, euparal or glycerine. The best results were obtained in euparal.

The measurements of the somatic chromosomes were made according to the methods used by FLOVIK (1938), with some modifications. At least ten of the best metaphase-plates in the root-tips from every diploid plant that was closely studied were measured in order to obtain its karyotype.

The drawings were done with the aid of a camera lucida, and the pollen fertility of all male plants was studied in acetocarmine-glycerine (1 : 1).

IV. CYTOLOGICAL PART I. MITOSIS.

1. RUMEX ANGIOCARPUS.

The basic number of *R.* sg. *Acetosella* is x = 7 as already mentioned. The group shows different degrees of polyploidy from the diploid to the octoploid state. The diploid species with 2n = 14 chromosomes is *R. angiocarpus*, as previously published by LÖVE (1941 b). The chromosomes are small, the longest pair of autosomes measuring 2,7 micra only, but they lend themselves fairly well to morphological analysis.

As the closely investigated material originated from only one locality on the Pyrenaean peninsula, the morphological analysis of the chromosomes cannot fully account for the variation in the morphology of the chromosomes in forms from different places within the distribution area of the species. This sample, however, showed one difference in the karyotype, which may indicate a possible variability of karyotypes in different forms somewhat like that reported before, for instance, in *Rumex Acetosa* and *R.* »*montanus*» (= *R.* »*arifolius*») from Japan (KIHARA and YAMAMOTO, 1931; ONO, 1935; YAMAMOTO, 1938, and elsewhere) and in a number of other species (cf. SHARP, 1934).

The different types of chromosomes in the mitotic phase of all the individuals were closely studied and measured on many plates of plants from Portugal. As in most of the cases the fixation was exceedingly successful with the chromosomes beautifully scattered and with sharply defined centromeres, most of the plants could be closely investigated and their chromosomes measured.

In accordance with the terminology of KIHARA and YAMAMOTO (1931), ONO (1935) and YAMAMOTO (1938) four types of somatic chromosomes are distinguished, i. e. *i*, *v*, *j*, and SAT-chromosomes, marked *T* by the Japanese investigators, but marked by the present writer as i^s. The SAT-chromosomes have only been found as *i* type chromosomes with satellites, but if *j* or *v* type chromosomes are found with satellites, they are to be marked j^s or v^s respectively. The characteristic form of the different types of chromosomes may be seen in Table 1.

The i^s type may, of course, be included in the *i* type (cf. ONO, 1935). The chromosomes of the different types may be different in length. Therefore, the present writer prefers to mark them also with numbers from 1 to 6. The denomination $i_{1,1}$ and $i_{6\ 6}$ then indicates that the *i* type autosomes are the longest and the shortest pairs respectively, and the double number indicates

TABLE 1. *The characteristics of chromosome types in Rumex.*

Type of chromosomes	Form	Centromere
i	stick-formed with a head on the proximal part	subterminal
i^s	*i* type with a satellite	subterminal
j	with unequal arms	submedian
v	with almost equal arms	nearly median

that they are found in symmetrical pairs. The denominations $i_3{}^s$ and i_3 or, e. g., j_4 and v_4 in one and the same karyotype indicate that the karyotype is asymmetrical in respect of the 3rd and 4th pairs of autosomes. The longest chromosome is marked with the number 1, the shortest with the number 6. The sex chromosomes are marked *X* or *Y*.

As only one symmetrical karyotype (74 individuals) and one asymmetrical (1 individual) have as yet been obtained within the species, it is not convenient to mark them in alphabetical or numerical order. Therefore, the two karyotypes theoretically found in the neighbourhood of Lisbon have been marked as karyotype P_1 and P_2. The karyotype of the asymmetrical type is marked P_{1+2}.

Karyotype P_1. — The chromosomes of a male individual are shown in Fig. 6 and those of a female individual in Fig. 7. The characteristic shape and types of the chromosomes can easily be seen in both the figures. The average length of the different types of the chromosomes obtained in the measurements is given in Table 2, and the diagrammatical idiogram of the types constructed on the basis of the values given in the table is shown in Fig. 8. The chromosomes in the diagram are marked according to the terminology given above. The chromosome formula of the karyotype P_1 is: $i_{5,\,5;\,6,\,6}$; $i^s{}_{4,\,4}$; $j_{3,\,3}$; $v_{1,\,1;\,2,\,2}$. This karyotype is symmetrical as all the autosomes are found in pairs.

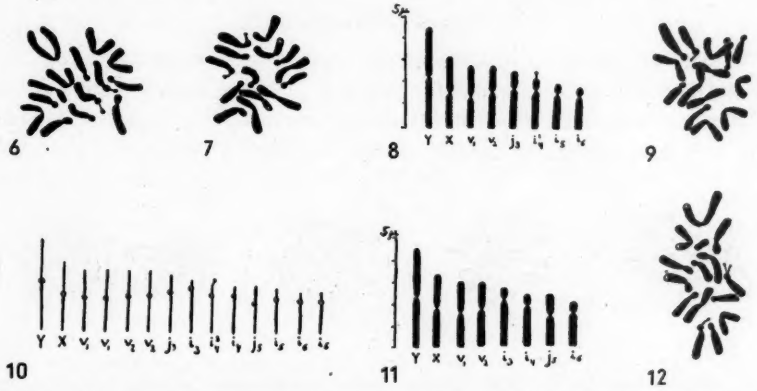
TABLE 2. *The average length of the different chromosome types in R. angiocarpus, karyotype* P_1.

Type:	*Y*	*X*	v_1	v_2	j_3	$i^s{}_4$	i_5	i_6
Length of the arms in micra:	2,0 2,4	1,4 1,6	1,3 1,4	1,1 1,6	0,7 1,8	0,1 0,3 1,7	0,3 1,6	0,3 1,5
Total length:	4,4	3,0	2,7	2,7	2,5	2,1	1,9	1,8

Karyotype P_{1+2}. — The chromosomes of one plant, *R 0583*, were found to differ from the type found in the other individuals examined, as mentioned above. The chromosomes of this male individual are

shown in Fig. 9. The average length of the different types of the chromosomes measured is given in Table 3, which is diagrammatically reproduced in Fig. 10. The chromosome formula of karyotype P_{1+2} is: $i_{3,4,5,6,6}$, i^{s}_{4}, $j_{3,5}$, $v_{1,1,2,2}$.

As seen in the diagram in Fig. 10 and in the karyotype formula of this type, it may have originated from a hybridization between karyotype P_1 and another karyotype, differing in the length of one pair of *i* type and one pair of *j* type chromosomes as well as in the absence of



Figs. 6—12. *R. angiocarpus*, chromosomes from somatic metaphase plates. — Figs. 6—8, karyotype P_1; Fig. 6, ♂; Fig. 7, ♀; Fig. 8, idiogram; Figs. 9—10, karyotype P_{1+2}; Fig. 9, ♂; Fig. 10, schematical idiogram; Fig. 11, theoretical idiogram of karyotype P_2; Fig. 12, ♂ of karyotype P_1 with a fragment. — Magnification × 2400.

the satellite on the i_4 chromosome. Theoretically, the karyotype formula of the other type must be: $i_{3,3;4,4;6,6}$, $j_{5,5}$, $v_{1,1;2,2}$, and diagrammatically it may have the appearance given in Fig. 11. This hypothetical karyotype will be denoted as a karyotype P_2. The unmixed karyotypes are always symmetrical, their hybrids always asymmetrical, as has also been observed in the writer's material of *R. Acetosa* (s. lat.) from Scandinavia (unpubl. data).

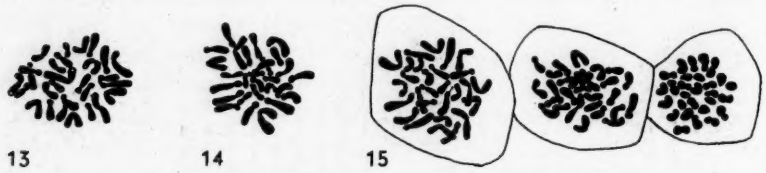
TABLE 3. *The average length of the different chromosome types in R. angiocarpus, karyotype* P_{1+2} *(sex chromosomes excluded).*

Type:	v_1	v_2	j_3	i_3	i^{s}_{4}	i_4	j_5	i_5	i_6
Length of the arms in micra:	1,3	1,1	0,7	0,3	0,1	0,3	0,7	0,3	0,3
	1,4	1,6	1,8	2,1	0,3	1,7	1,3	1,6	1,5
					1,7				
Total length:	2,7	2,7	2,5	2,4	2,1	2,0	2,0	1,9	1,8

Karyotype P_1 with a fragment. — In one male individual, *R 0597*, a fragment was detected in the mitotic as well as in the meiotic divisions. The karyotype of this plant was identical with karyotype P_1 and the fragment is possibly to be regarded as genetically inert, as no influences from it could be detected. Perhaps it is the result of previous hybridization between two karyotypes or individuals differing in an inversion. A somatic metaphase plate from this individual is shown in Fig. 12.

2. RUMEX TENUIFOLIUS.

The chromosome number of this species is $2n = 28$, as previously published by LÖVE (1940 a, b, 1941 a, b). The chromosomes are of the same four main types as described above for *R. angiocarpus*, but as they are small and almost always somewhat crowded, the writer



Figs. 13—15. *R. tenuifolius*, chromosomes from somatic metaphase plates. — Fig. 13, ♂; Fig. 14, ♀; Fig. 15, three cells side by side showing differences in spiralization. — Magnification × 2100.

was not able to determine the exact karyotype of any individual. Studying the most characterizing types of chromosomes as, e. g., SAT-chromosomes and the longest *j* chromosomes, four chromosomes of the same type were hardly ever observed and measured. The present writer therefore supposes that pure karyotypes will practically speaking never be found within this tetraploid species. The part this plays in the evolution of the species will be discussed later on in connection with a discussion on the kind of polyploidy met with in the subgenus. *Only the exact tetraploid number $2n = 28$ has been counted in my material*, no individual showing deviating numbers. Figs. 13 and 14 show the somatic chromosomes from a male and a female individual respectively.

It may be of some interest to mention here that in one individual of this species, originating from the neighbourhood of Moscow, three cells side by side in the same root showed striking differences in the degree of spiralization (Fig. 15). All the cells showed the same stage of mitosis, i. e. metaphase, but the cell nearest the outside of the root (to the left in Fig. 15) had normal chro-

mosomes when the other two showed differences in the size of all the chromosomes. Somewhat similar cases have been reported, for instance, by BLACKBURN (1933), in different plants of *Spergularia*, by HORN (1938) in *Papaver* and by D. LÖVE (1942 b) in the root-tips of *Silene livida*. It is practically out of the question, at least in the case of *R. tenuifolius* and *S. livida*, that this could always be due to external influences, as, e. g., to the specimens' having been dry when fixed, as observed by HORN (l. c.), since the differences are met with in one and the same root-tip. This phenomenon may, however, be influenced by the supply of air or the nourishment of the different cells.

3. RUMEX ACETOSELLA S. STR.

The chromosome number of this species has been determined by ROTH (1906), who counted the haploid number n = 16. It is, however, quite possible that this inexact number may have been determined from material of the tetraploid species mentioned above, as it is very common in the western parts of Germany. Unfortunately, ROTH gave

16 17 18

Figs. 16—18. Somatic metaphase plates. — Fig. 16, *R. Acetosella* s.str. ♂; Fig. 17, *R. Acetosella* s.str. ♀; Fig. 18, *R. graminifolius*. — Magnification × 2100.

no account of the type of the plants studied by him, nor did he refer to any herbarium specimens of the material investigated.

The exact chromosome number of the species was determined by KIHARA (1925, 1927 a, b, 1929), who found the hexaploid number 2n = 42 in the females and 2n = 41, 42 and 43 in the males. MEURMAN (1925 a, b) counted the number n = 20 and 21 in the meiosis of a male individual. He concluded that the somatic chromosome number of the male individuals was 2n = 41 and in the females 2n = 42. KIHARA (1927 b) investigated the meiosis of fifteen male plants and found the number 2n = 42 in most of them, but the number 2n = 41 occurred in three and the number 2n = 43 in one individual. According to KIHARA (1927 a, b, 1929) the normal chromosome number of both male and female individuals of *R. Acetosella* s.str. is 2n = 42, although some minor deviations may occur in the male plants. Later reports of the chromosome number of this species are given by ONO (1930), who found 2n = 41 chromosomes in an intersexual plant, and by JENSEN (1936 a, b), who determined the number n = 21 or 22. The

latter determination, however, must be taken with some reservation, according to what JENSEN states about the occurrence of sex chromosomes in his material.

The present writer has counted the chromosome number in the root-tips of a large number of male and female individuals from natural localities and in a large number of hybrids between material from different places. *The chromosome number in most of the plants proved to be* $2n = 42$ *both in male and female individuals* (Figs. 16—17). In some few plants of both sexes some deviating numbers were counted. *The number* $2n = 41$ *is found in 5 % of the males and 4 % of the females and in about 1 % of the males and females the chromosome number was* $2n = 43$. In a few individuals of both sexes the exact chromosome number could not be determined because of the chromosomes' being too crowded in the metaphase plates.

The writer was not able to determine the exact karyotype of any plant of *R. Acetosella* s.str. He could, however, establish that at least the most typical autosomes were never found to number six, as might be expected if the karyotypes were unmixed, and in most of the cases only one pair of the identifiable types could be observed with any certainty.

4. RUMEX GRAMINIFOLIUS.

The somatic chromosome number of this arctic species is the octoploid one, i. e. $2n = 56$ chromosomes (Fig. 18), as previously demonstrated by LÖVE (1941 b). As the chromosomes are small and crowded, the number could not be exactly determined in many plates, and the morphology of the chromosomes was not studied in detail. Their size and form, however, are the same as are met with in the three other species of the subgenus described above.

V. CYTOLOGICAL PART II. MEIOSIS (AUTOSOMES).

1. MEIOSIS IN RUMEX ANGIOCARPUS.

A. REGULAR TYPE.

The meiosis of thirty-one male individuals of the diploid species has been scrutinized in order to detect meiotic irregularities. Of these individuals six showed some meiotic disturbances, the other twenty-five manifesting only fully regular meiosis in about one thousand pollen mother-cells studied from each plant.

A distinguishing feature in the flowers of *Rumex* is the very long pre-meiotic resting stage of the archespores. At this stage the volume of the cell and of the nucleus increases and the stamens grow to double the size, but the prophase begins under normal climatic conditions at the earliest a week or so after the last pre-meiotic division.

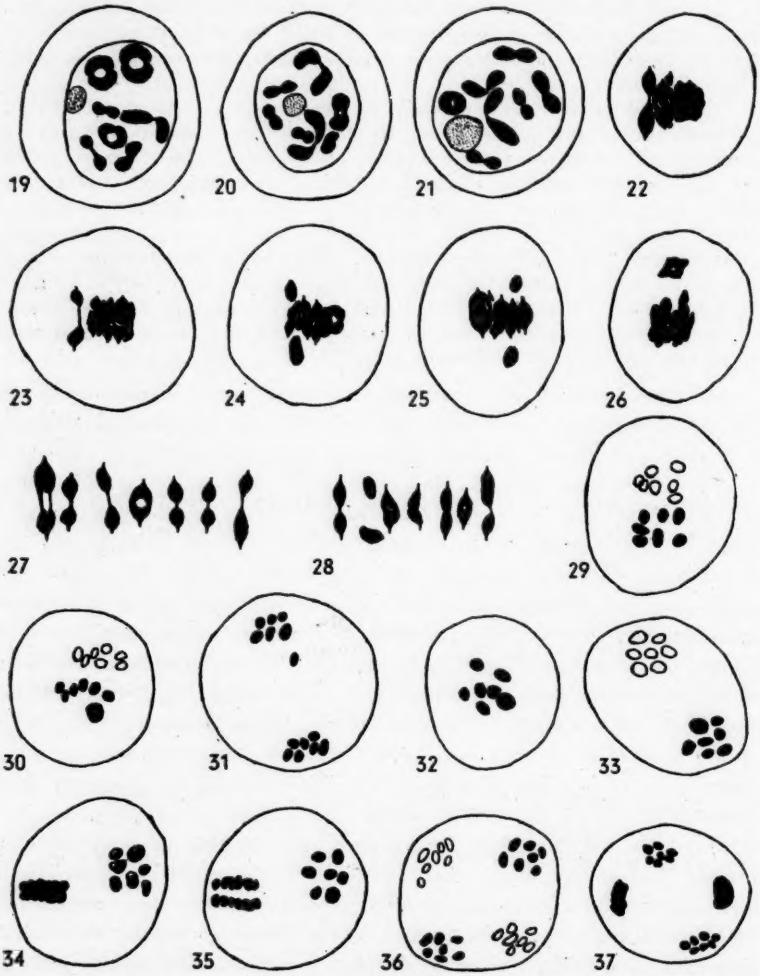
In the twenty-five normal plants the prophase was of the normal type. The fixations used were not very well adapted for closer analysis of the prophase stages, and therefore only a very few small observations were made without drawings.

In early prophase the chromosome threads are very elongated and difficult to observe, but as the nucleus advances into prophase simple undivided long slender spiral threads can be seen. These threads seem to be somewhat polarized, and they contract to a »bouquet» when the zygotene pairing begins. The pairing begins at the pole, as reported by KIHARA (1927 b) in *R. Acetosella* s.str. Unfortunately, the different threads could not be more closely analysed in the material during pachytene-zygotene. At the end of diplotene the threads have grown thicker and shorter and now they are more uniformly distributed throughout the nucleus. The whole pachytene stage appears to be of rather considerable length, judging from its frequency and from the different size of the flowers and stamens where it is found.

The diakinesis leads directly from the gradual contraction of the late diplotene threads. The gemini are of about the same size as in metaphase-I in later stages of diakinesis (Figs. 19—21).

The metaphase plate is then formed in the usual way. In most of the cells studied the plates were fully normal with the sex chromosomes on the one side (Figs. 22—23). In some cases, however, the sex chromosomes seem to separate somewhat precociously (Figs. 24—25), although it is not impossible that this is no real precocious separation (cf. DARLINGTON, 1937; BĚLAŘ, 1929, and others). Non-congression of the sex chromosomes, however, is undoubtedly met with in a few cells, as has previously been found by some other writers, e. g., HEITZ (1925) and D. LÖVE (unpubl.) in *Melandrium* and KOLLER and DARLINGTON (1934) in *Rattus*. One plant, *R 0531*, showed a low frequency of non-congression of one pair of autosomes, which formed an accessory plate between the pole and the normal metaphase plate (Fig. 26) (cf. DARLINGTON, 1937). This phenomenon may be due to some external factors, but it might possibly prove to be a result of some genic differences or of a decrease of the repulsion-power of the centromeres. All the autosome bivalents studied in the accessory plates were small ring-bivalents with a somewhat delayed terminalization, which possibly indicates a reduced repulsion-power. When the sex chromosomes fail to orientate it may very likely be due to the long distance between the centromeres (cf. DARLINGTON, 1937; RICHARDSON, 1936).

The seven metaphase bivalents, drawn separately, are shown in Figs. 27—28. Their differences in size are in accordance with the differences found in the mitotic phase, although the different types cannot be pointed out with any certainty. In Fig. 27 all the chromo-



Figs. 19—37. Meiosis in *R. angiocarpus*, normal type. — Figs. 19—21, diakinesis; Figs. 22—26, metaphase-I, Figs. 27—28, metaphase-I chromosomes drawn separately; Figs. 29—32, anaphase-I; Figs. 33—34, metaphase-II; Figs. 35—36, anaphase-II; Fig. 37, telophase-II. — Magnification × 2100.

somes are paired, but one pair of possible univalents is shown in Fig. 28.

The normal anaphase-I is reproduced in polar view in Figs. 29—30, both giving the distribution 7 : 7. Fig. 31 shows an anaphase-I with one chromosome somewhat delayed. In Fig. 32 the six autosomes and one of the sex chromosomes are shown in polar view of one of the anaphase-I plates.

The short interphase stage between the first and second division is interrupted by the second metaphase (Figs. 33—34) and the second anaphase (Figs. 35—37) and telophase. The second division is of very short duration, compared with the other stages of the meiosis.

B. TYPE WITH INVERSION.

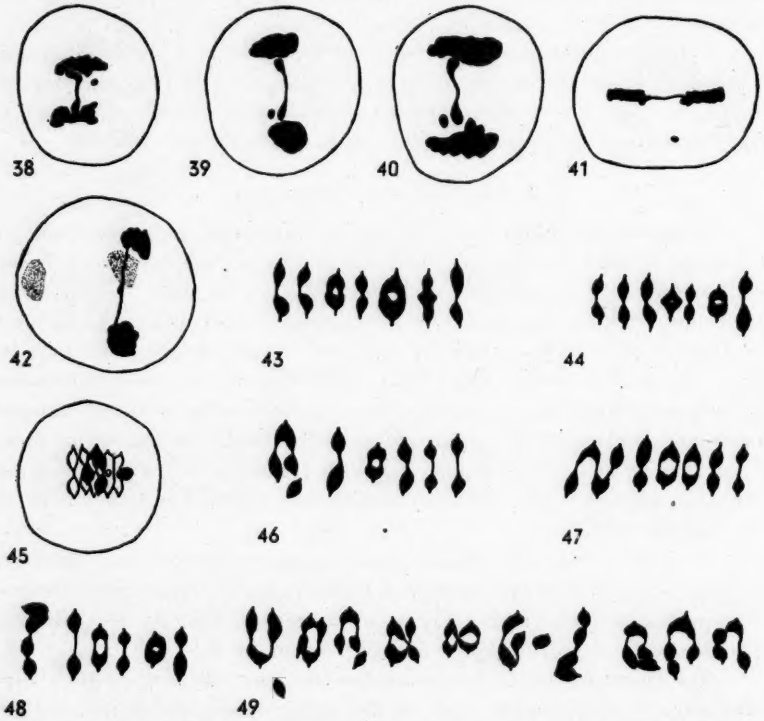
Dicentric chromatids and acentric fragments were observed in the plant *R 0548*, both in the first and in the second division. Three cells with such irregularities are shown in Figs. 38—40. In Fig. 41 a persistent bridge is shown in the metaphase-II, and in Fig. 42 another bridge in the second division is depicted. The bridges illustrated in Figs. 38—41 may be the results of a single crossing-over in an inversion heterozygote forming the well-known inversion bridge with a fragment most commonly met with in literature. The bridge in Fig. 42 is, however, formed as a loop chromatid with a fragment in the first division (cf. DARLINGTON, 1937). The fragment may or may not be visible in the second division.

In some of the interphases studied a micronucleus was observed, and in a number of the second telophases extra nuclei were found. The pollen »tetrads» showed some irregularities, such as, for instance, pentads with very different sizes of »tetrad» cells.

The frequency of the inversion bridges was very low, as they were met with in only about 1,5 % of the three thousands pollen mother-cells examined. This must be due to the inverted segment being very small or located in a part of the chromosome where the crossing-over frequency is very low. As the first alternative seems to be somewhat ruled out by the size of the fragment, the writer supposes that the inversion met with in this plant is to be found in the shorter arm of either the *j* chromosome or one of the *i* chromosomes. In these chromosomes the chiasma frequencies are low and there is every probability that crossing-over is much more frequent in the longer arm (cf. below). Some other interpretations are, however, possible (cf. DARLINGTON, 1937).

The somatic chromosomes of this plant showed that it belonged to the symmetrical karyotype P_1. Thus, *inversions may be found within the symmetrical karyotypes.*

Unequal bivalents have often been observed in inversion heterozygotes (cf. DARLINGTON, 1937; SANSOME and PHILP, 1939). Closer



Figs. 38—49. Meiosis in *R. angiocarpus*. — Figs. 38—44, type with inversion; Figs. 38—40, bridges with fragments at anaphase-I; Fig. 41, a persistent bridge at metaphase-II; Fig. 42, a bridge at anaphase-II; Figs. 43—44, metaphase-I chromosomes, drawn separately; Figs. 45—49, type with segmental interchange; Fig. 45, metaphase-I; Figs. 46—48, metaphase-I chromosomes, drawn separately; Fig. 49, associations of three or four chromosomes from different metaphase-I groups. — Magnification × 2100.

investigations were therefore made of the bivalents of a number of cells. No inequality was, however, met with in the autosome pairs examined (cf. Figs. 43—44).

It is worth while emphasizing here that inversions have in fact

been detected in all the species of *Drosophila* where they were looked for (cf. DOBZHANSKY, 1937), and they will without doubt be found in at least most of the allogamous plants. Failure to detect chromatin bridges may be due to failure or lowness of chiasma frequencies in the inverted segment as found in *R. angiocarpus*, and therefore it must not always be regarded as proof that the organism is free from inversions. The author assumes that at least a few of the individuals of this species investigated and found to have only regular meiosis might still be heterozygous for inversions.

The effect of inversions on the pollen fertility in the plant kingdom in general and in *R.* sg. *Acetosella* and other dioecious plants in particular will be discussed in greater detail later on in connection with the discussions of the meiotic irregularities in relation to the fertility of pollen grains.

C. TYPE WITH SEGMENTAL INTERCHANGE.

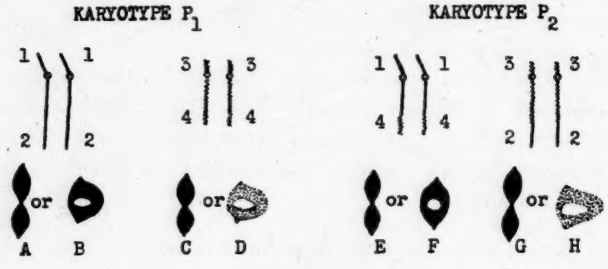
Segmental interchange between non-homologous chromosomes could be demonstrated in one plant, *R 0583*. It was shown above that this plant may be regarded as a hybrid between two different karyotypes, karyotype P_1 found in the other individuals studied, and karyotype P_2, which, theoretically, may be found in the neighbourhood of Lisbon.

The prophase stages of this plant were not examined, but the writer scrutinized the first metaphase configurations very closely in 40 cells. In 24 cells the configuration was $1_{IV} + 5_{II}$ (the *XY*-bivalent excluded), in 4 cells it was $1_{III} + 5_{II} + 1_{I}$, and 12 cells showed only bivalents. Thus, an *association of four chromosomes was met with in 60 % of the cells studied*, and in 30 % of the cells only bivalents were observed. The association of four chromosomes was represented by rings or chains. Of the 24 associations studied, 11 belonged to the ring category, all of the other being chains. Only one ring was a zig-zag ring, three chains showed a zig-zag arrangement, but all the other configurations were of the non-zig-zag type. All the associations of three chromosomes were of the chain type and they were all of the U-shape shown in Figs. 45, 46 and 49.

As it was extremely difficult to analyse the cells with the zig-zag configurations, the possibility is not excluded that the frequency of the zig-zag chains and rings, as well as the frequency of the associations of four chromosomes as a whole, might perhaps prove to be more frequent if a large number of cells were analysed.

The type of »trivalents» with univalents met with is shown in Fig. 45, and a complete group of chromosomes from another cell with a »trivalent» is separately drawn in Fig. 46. In the first-mentioned plate the univalent lay in the middle of the plate, in the second plate, drawn separately, it was found to be somewhat outside.

Two metaphase plates with associations of four chromosomes are



	2 x-ta	3 x-ta	4 x-ta
3.4 - 4.1 - 1.2 - 2.3	-	4-chain	4-ring
4.1 - 1.2 - 2.3 + 3.4	3-chain + 1_I	-	-
1.2 - 2.3 and 1.4 - 4.3	2_{II}-rod	-	-
2.1 - 1.4 and 4.3 - 3.2	2_{II}-rod	-	-

Fig. 50. Origin of the plant with segmental interchange. Explanation in the text.

shown in Figs. 47—48. Both belong to the chain category, the one being a zig-zag chain, the other a non-zig-zag chain. Some of the associations met with in different cells are separately drawn in Fig. 49. There it can be seen that clear differences between the chromosomes in the association may be observed in the meiotic metaphase, as is also found in the measurements of the somatic chromosomes. The univalents invariably belong to the smaller pair and must be identical with the i_5 chromosome (cf. below). When the con-

figuration is zig-zag, one of the large and one of the small chromosomes of the configuration will go to the same pole, as may be seen in, for instance, the 4th and the 5th configurations from the left in Fig. 49.

In anaphase-I, disjunction proved to be regular with 7 chromosomes to both the poles in only 49 % of the cases studied, the other *51 % showing the distribution 6 : 8.* The first-mentioned category includes, however, a number of cases of genetical non-disjunction, as both the chromosomes of each »pair» must frequently go to one and the same pole. Therefore, *more than $^2/_3$ of the gones may be assumed to be the result of a numerical and genetical non-disjunction.*

As shown above in connection with the karyotype analysis, this plant may be regarded as a hybrid between two different karyotypes. It could be demonstrated that the interchanged segments differ in size. The segment transferred to the *i* chromosome is rather long, but the segment on the *j* chromosome must be of very small size. The two pairs of chromosomes which form the association in *R 0583* are shown schematically in the diagram in Fig. 50. Firstly, the two pairs are shown in the symmetrical karyotypes P_1 and P_2. In karyotype P_1 the *j* chromosomes are marked as 1.2 and the *i* chromosomes as 3.4. The *j* chromosomes may form the bivalents *A* (rod, most frequent) and *B* (ring, infrequent), and the *i* chromosomes form the rod-bivalent *C*, and, theoretically, the ring-bivalent *D*. The ring-bivalent *D* has, however, never been observed in my material. In karyotype P_2 the *j* chromosomes are marked as 1.4 and the long *i* chromosomes as 3.2. In this type the *j* chromosomes may form the rod-bivalent *E* and the ring-bivalent *F*. The *i* chromosomes form the rod-bivalent *G*, and possibly, in a very low frequency, the ring-bivalent *H*.

The chromosomes in the hybrid are given in the figure under the above-mentioned karyotypes. The *j* chromosomes are 1.2 and 1.4, the *i* chromosomes 2.3 and 3.4. Their theoretical associations are given in the table below the figure. When forming an association of four the highest number of chiasmata is four, resulting in a ring. Since as a rule the *i* chromosomes fail to form a chiasma at the proximal end (marked as 3), frequently only three chiasmata will be formed. This makes it easy to understand the relative commonness of the chain associations of four chromosomes. The unexpected high frequency of univalents is easily explainable as the result of failure to pair between the proximal part of the *i* chromosome (3) and in the small interchanged segment (4). The univalent must, therefore, always be identical with the normal i_5 chromosome from karyotype P_1. Sometimes (in 30 % of the cases studied) two bivalents will be formed as a result of a failure to pair in the middle of the chain, either between the segment 4 or the ends 3 and 1. The bivalents 2.1—1.4 and 4.3—3.2 or 1.2—2.3 and 3.4—4.1 will then be formed. Only two chiasmata will be formed. If the bivalents 2.1—1.4 and 4.3—3.2 are formed, they must be unequal. In all but two of the cells with only bivalents no unequal pair was found (except the sex chromosome pair), which indicates that most frequently the distal ends form the chiasma of these bivalents giving no visible inequality. This agrees, in fact, with the suggestion

made above that the proximal ends of the *i* and *j* chromosomes most frequently form no chiasma.

Theoretically, only one chiasma, or none at all, will be formed a few times, giving one bivalent and two univalents or four univalents respectively. This has, however, never been observed in my material.

D. TYPE WITH A FRAGMENT.

As mentioned above, one plant proved to contain a fairly small fragment in the mitotic metaphases. The behaviour of this fragment was studied to some extent during meiosis. It was found that in most of the metaphase-I plates it can be observed beside the bivalents (Fig. 51), but it was also observed sometimes outside the plate (Figs. 52—53). It was always found free and never associated with other chromosomes, which affords strong evidence that it represents only a fragment. At the first anaphase the fragment is almost invariably included in one of the groups, but in a few pollen mother-cells it was observed as »lagging» (Fig. 54), or else it was dividing (Fig. 55). The second division was not closely examined, but in consequence of the somewhat regular inclusion of the fragment in one of the two interphase nuclei it is very likely to be transmitted to about 50 % of the male gones.

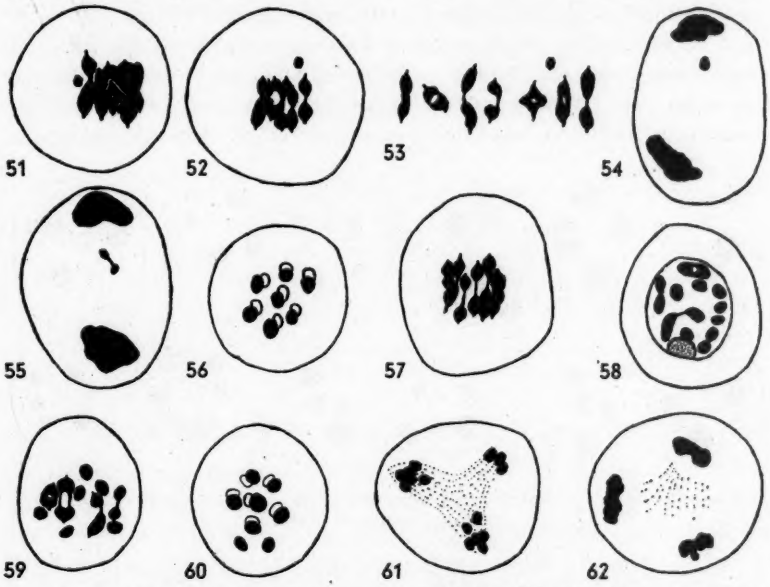
E. TYPE WITH PARTIAL ASYNAPSIS.

Plant *R 0581* showed low but evident decrease in the meiotic pairing. Unfortunately, only a small number of diakinesis and metaphase-I plates could be perfectly analysed; nevertheless it could be demonstrated that in about 80 % of the cells the pairing is fully normal. The other 20 % showed asynapsis in from one to six pairs of autosomes, the configuration $4_{II} + 6_{I}$ being the most frequent (cf. Table 4). The sex chromosomes were always found as a rod-bivalent.

A normal metaphase plate with 7_{II} is shown in polar view in Fig. 56 and in side view in Fig. 57. A diakinesis with $4_{II} + 6_{I}$ from the same plant is shown in Fig. 58, and in Figs. 59 and 60 metaphases with univalents are to be found in side and in polar view respectively.

In the partial asynaptic individual, moreover, some low frequency of tripolar spindles was observed, as has been found before, e. g., in the asynaptic *R. Acetosa* by YAMAMOTO (1934) as well as in plants with otherwise normal meiosis (HEITZ, 1925, for *Melandrium*; OKABE, 1929, for *Psilotum*). Tripolar spindle has been reported after cold-treatment or after treatment with some chemical agents, as well as after hybridization in some plants and animals (cf. DARLINGTON, 1937). Only in

the fern *Psilotum* is it found in almost all the spore mother-cells of a tetraploid species (OKABE, 1929). In *R. angiocarpus* it is observed only in that individual with partial asynapsis. Two cells with a tripolar spindle are depicted in Figs. 61—62. Cells with three interphase nuclei



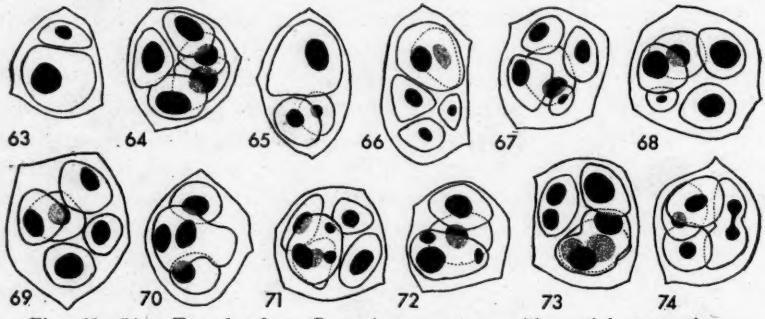
Figs. 51—62. Meiosis in *R. angiocarpus*. — Figs. 51—55, type with a fragment; Figs. 51—53, metaphase-I; Figs. 54—55, anaphase-I; Figs. 56—62, type with partial asynapsis; Fig. 56, metaphase-I, polar view, 7_{II}; Fig. 57, metaphase-I, side view, 7_{II}; Fig. 58, diakinesis, $4_{II} + 6_I$; Fig. 59, metaphase-I, $4_{II} + 6_I$; Fig. 60, metaphase-I, $6_{II} + 2_I$; Figs. 61—62, tripolar spindle at anaphase-I and telophase-I. — Magnification × 2100.

TABLE 4. *Metaphase-I configurations in R. angiocarpus, type with partial asynapsis.*

Configuration	Number of cells
7_{II}	158
$6_{II} + 2_I$	4
$5_{II} + 4_I$	6
$4_{II} + 6_I$	19
$3_{II} + 8_I$	10
$2_{II} + 10_I$	2
$1_{II} + 12_I$	1
$0_{II} + 14_I$	0
	Total: 200 cells

of different sizes were fairly frequent. They are probably the results of the tripolar spindle.

The pollen »tetrads» of the asynaptic plant showed some interesting disturbances. The most common ones were dyads with one large and one small cell (Fig. 63), pentads with almost equally large cells (Fig. 64) or with large and small cells combined (Figs. 65—69). In exceptional cases the »tetrads» had cells with two or more nuclei in one (Figs. 70—72) or more cells (Fig. 73). Some cells with a nucleus recalling a restitution nucleus were also observed (Fig. 74).



Figs. 63—74. »Tetrads» from *R. angiocarpus*, type with partial asynapsis. — Magnification × 1350.

F. TYPE WITH A LOW FREQUENCY OF FAILURE OF THE SECOND DIVISION.

In one plant failure of the second division was observed in between 1 and 2 % of the pollen mother-cells. This phenomenon, however, was not thorougly examined when it was observed at first, but since then the slides have become so dark that a closer analysis is impossible. The results of the failure of the second division were dyads with equal cells.

G. TYPE WITH »FUSION» OF THE TELOPHASE-II »TETRADS».

The individual *R 0544* showed a low frequency (about 1 %) of »fusion» of two and two of the cells immediately after the second telophase but before the walls were formed. The first and second divisions were fully normal, but when most of the telophase-II products formed the walls, some cells increased in volume without forming a wall and arranged themselves two and two together. The cells fuse (Fig. 75) and the nuclei of one of them stroll into the cavity of the other (Fig.

76). No later stages of this »fusion» were observed, but a low frequency of giant pollen grains with one very large nucleus indicates that all the nuclei may fuse into one in the first mitotic division of the pollen grain.

The cause of this phenomenon may very likely be of a physiological nature; possibly it is due to some disturbances in the behaviour of the spindle and the phragmoplast. It is not to be confused with the phenomenon of »cytomixis», which probably takes place in the pre-meiotic divisions.

Figs. 75—76. Two »cells» with »fusion» of the telophase-II »tetrads». — Magnification × 1350.

H. CHIASMA FREQUENCIES IN RUMEX ANGIOCARPUS.

The chiasma frequencies were examined in metaphase-I of the three male individuals *R 0531*, *R 0553* and *R 0595*, all showing fully normal meiosis, in *R 0548*, the type with inversion, and in *R 0583*, the plant with a segmental interchange. As a rule the bivalents are united by one or two chiasmata, which are always found to be fully terminalized at metaphase. As the affinity of the sex-linked heterochromosomes is different from the autosomes, they are not included in the calculations on the chiasma frequencies. The results obtained from the study of the twelve autosomes are listed in Table 5.

TABLE 5. *Chiasma frequencies in the autosomes of different male plants of R. angiocarpus.*

Field number	Total number of x-ta per cell 5	6	7	8	9	10	n	M ± m	x-ta per chromosome
R 0531	1	4	9	12	5	—	31	7,52 ± 0,18	0,626 ± 0,015
R 0553	—	5	8	13	4	—	30	7,40 ± 0,15	0,615 ± 0,013
R 0595	—	5	11	11	3	—	30	7,40 ± 0,16	0,617 ± 0,013
R 0548 (inversion)	—	6	8	12	4	—	30	7,47 ± 0,17	0,623 ± 0,014
R 0583 (segm. interch.)									
(total):	—	1	6	16	13	4	40	8,33 ± 0,15	0,694 ± 0,013
(with 6_{II}):	—	1	3	7	1	—	12	7,67 ± 0,22	0,639 ± 0,018
(with III and IV):	—	—	3	9	12	4	28	8,61 ± 0,16	0,717 ± 0,013

The most common type of bivalents is the rod with only one chiasma. This type is common to the *i* and the i^s chromosomes and mostly also to the *j* chromosomes with one short and one long arm, although it happens that the *v* chromosomes also fail to form more than one chiasma.

The three normal individuals differ somewhat from each other in total chiasma frequencies, but the differences are not statistically significant. The chiasma frequency of the type that is heterozygous for an inversion falls within the limits of the values obtained in the normal plants. The average chiasma frequency in all the autosomes of the normal karyotype P_1 was 7,44 per cell, corresponding to a mean value per chromosome of 0,620. Hence, about 66 % of the autosomes form only one chiasma, a fact strongly indicating that in most of the cells only in exceptional cases will both the ends of the *j* chromosomes and practically never both the ends of the *i* and i^s chromosomes be united with a chiasma. In actual fact, never more than three ring-bivalents were observed in the same cell, and then they must be formed by the two pairs of *v* chromosomes and most possibly also of the *j* chromosome pair.

In the karyotype P_2, which hypothetically may exist, somewhat higher chiasma frequencies may be expected, as the difference between the two arms of the *j* chromosomes is not as large as that found in the karyotype P_1. In the plant that is heterozygous for the segmental interchange, however, no differences in the chiasma frequencies from the normal karyotype P_1 may, theoretically, be detected in the cells with 6_{II}, as the short *j* chromosome is not found there in the homozygous stage. Theoretically, the chiasma frequencies of karyotypes differing in the number of *v* chromosomes must show significative differences from each other. This problem cannot be examined at present in *R. angiocarpus* for the reason that material has not yet been obtained from different localities with different karyotypes. It will, however, be closely studied in the author's material of different karyotypes of the Scandinavian forms of *R. Acetosa* (s.lat.).

As to the differences in chiasma frequencies between normal individuals and types with associations of more than two chromosomes, two different hypotheses are met with in literature. CATCHESIDE (1933) has suggested that in *Oenothera* there is a general tendency for the chiasma frequency to be lower in the rings than in the bivalents. MÜNTZING (1937), however, showed that in *Dactylis Aschersoniana* the reverse is the case. These differences may be due to the fact that in

Oenothera most of the bivalents form two or more chiasmata, whereas in *Dactylis* only slightly more than half of the metaphase chromosomes are found to have two chiasmata. This may also be due to the fact that the ring-forming chromosomes in associations of more than two chromosomes in *Dactylis* were of the rod-bivalent type, normally forming only one chiasma at metaphase-I. The results obtained by STEBBINS and ELLERTON (1939), who detected lower chiasma frequency in the associations than in the bivalents in two clones of *Paeonia californica* and the reverse in a third clone, may be interpreted as being due to karyotype differences within the species or to the associations in the first case being formed from only ring-bivalents, in the second case from only rod- or both rod- and ring-bivalents.

As shown above, the chromosomes forming the ring of four in *R. angiocarpus* belong to the *i* and *j* types, mostly forming only one chiasma per bivalent. It was therefore suggested that this case must belong to the same category as *Dactylis*, i. e. a positive correlation between the chiasma frequency and the occurrence of associations of more than two chromosomes may be expected.

In the plant with interchange the writer was able to examine the chiasma frequencies in 40 cells. The values obtained are listed in Table 5. The average frequencies of chiasmata per cell and per chromosome, calculated from the total sum of all the 40 cells, were significantly higher than in the normal individuals, the values found per cell being 8,33 and per chromosome 0,694. These results are in full accord with what might be expected if the hypothetical interpretation made above on the basis of the type and form of the mitotic as well as the meiotic chromosomes involved in the association were right.

In spite of the increased chiasma frequency in the type with segmental interchange, the chiasma frequency met with in cells with only bivalents must be the same as in normal individuals of karyotype P_1, if the above interpretation is correct. Accordingly, the chiasma frequency of the 12 cells with only bivalents studied in *R 0583* are calculated separately in Table 5. There it can be seen that the values obtained for these twelve cells show no differences from the normal individuals, as the mean value per cell observed was 7,67 and per chromosome 0,639, or only a very little higher than in *R 0531* with fully normal meiosis.

The real chiasma frequencies for the cells with associations of more than two chromosomes were obtained from the 28 cells with such associations. The means of chiasmata per cell as well as per chro-

mosome were shown to be somewhat higher than those calculated for all the 40 cells. This lends further support to the above hypothesis.

It is worth while to point out here that, although this analysis of the chiasma frequencies in *R. angiocarpus* supports the results obtained by MÜNTZING (1937) in *Dactylis Aschersoniana*, some clear differences are found between these two cases. *Rumex* and *Dactylis* coincide to the extent that the frequency is higher in the associations than in the bivalents. They differ, for instance, in the total average chiasma frequency of the interchange type of *Rumex* being higher than in the normal individuals, whereas in *Dactylis* this value is almost the same as is observed in the normal plants. According to MÜNTZING (1937), his results in *Dactylis* might be interpreted as being »due to a negative correlation of chiasma frequencies, being an expression of a tendency to unitary control of the chiasma number per cell». In *R. angiocarpus*, however, the higher values obtained for the ring-forming plant are considered to be due merely to the fact that the *i* and *j* chromosomes normally forming only one chiasma per bivalent and 0,5 chiasma per chromosome will form about 0,75 or 1,0 chiasma per chromosome when forming a chain or ring of four. The chiasma frequencies of the other *i* and *v* chromosomes are the same as normal; moreover, the cells forming only bivalents show no differences from the normal state, although the *i* and *j* type chromosomes of the association show incomplete homology, as normally these chromosomes form only rod-bivalents.

2. MEIOSIS IN RUMEX TENUIFOLIUS.

All the individuals, both the absolutely wild ones and their experimental hybrids, belonging to this tetraploid species showed the exact tetraploid chromosome number $2n = 28$. Nevertheless, meiotic irregularities acting as bridges with fragments in anaphase-I, multivalents or univalents, were found to be frequent, and some times at least the distribution of chromosomes at anaphase-I must be 13 : 15 instead of the normal one 14 : 14. The pollen fertility is, however, apparently good. The regularities in chromosome number found within this species may be due to zygotic lethality of forms with deviating numbers, a suggestion supported by the apparent fertility of the pollen grains in the diploid type with segmental interchange described above, as well as by some other observations mentioned below.

The meiotic behaviour of some male plants from different localities will be described below, and the meiosis of individuals obtained

from crosses between plants from different localities is also described. The prophase stages have not yet been closely studied in any individual.

A. R 077—5, FROM KRISTINEBERG.

This individual from Kristineberg, Bohuslän, is of the type *R. tenuifolius* var. *minimus*: a repent, small plant with almost thread-like leaves. The height of its longest stems is only about 10 cm. and its pollen fertility is good.

The studies of meiosis in this plant have been made at the metaphase-I and later stages. The following results have been obtained:

a. *Metaphase-I configurations.* — In the first metaphase groups the following configurations of the 20 cells completely analysed were observed in side view:

Configuration	Number of cells
$1_{VI} + 2_{IV} + 7_{II}$	1
$1_{V} + 1_{IV} + 1_{III} + 8_{II}$	1
$3_{IV} + 8_{II}$	1
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	2
$2_{IV} + 10_{II}$	2
$1_{IV} + 12_{II}$	9
14_{II}	4
	Total: 20 cells

It is evident from this table that quadrivalents are found in 80 % of the cells studied and that more than one multivalent are met with in 35 % of the cells. Two quadrivalents are found in two cells and three quadrivalents (not including the sex chromosomes) are observed in one cell. As shown in connection with the studies of *R. angiocarpus*, the chiasma frequency per chromosome is very low, and the *i* type, i^s type and *j* type chromosomes almost invariably form only 0,5 chiasma per chromosome. The studies of the somatic chromosomes of *R 077—5* showed that about half of the chromosomes in this individual belonged to the *v* type, which was shown to have about 1 chiasma per chromosome in *R. angiocarpus*. The maximum number of quadrivalents expected per cell in an autopolyploid of this type was therefore 3_{IV}, the sex chromosomes then forming only bivalents, as observed in 1 cell. Some of the *v* chromosomes of each size must, however, have been somewhat different, as the configuration $1_{IV} + 12_{II}$ was so much more frequent than the others and as the configuration $2_{IV} + 1_{III} + 8_{II} + 1_{I}$ was observed in two cells. This assumption is moreover to some extent supported by the fact that about 35 % of the quadrivalents observed formed a chain instead of a ring. The chromosome configurations

found in two cells are drawn separately in Figs. 77—78 and a polar view of a metaphase group with two quadrivalents is shown in Fig. 79.

As shown in the table, configurations of more than four chromosomes were found in two cells, i. e. in 10 % of the cases examined. *This must be due to heterozygosity for segmental interchange between different pairs of chromosomes*, as previously found in some other tetraploid plants as, e. g., *Anthoxanthum odoratum* (KATTERMANN, 1931), *Aucuba japonica* (MEURMAN, 1929 a), *Rosa relicta* (ERLANSON, 1931), *Campanula persicifolia* (GAIRDNER and DARLINGTON, 1931; DARLINGTON and GAIRDNER, 1937), and others. As the heterozygosity for segmental interchange met with in the diploid species was shown to be due possibly to the fact that the plant was a hybrid between two karyotypes differing in an interchange, it is not impossible that the plant *R 077—5* might be heterozygous for more than one related karyotype. Some multivalent autosomes from different cells are reproduced separately in Fig. 80.

It is worth while here to emphasize that in 15 cells out of the 20 studied the sex chromosomes were observed as bivalents, and in 5 cells only did they form a quadrivalent chain.

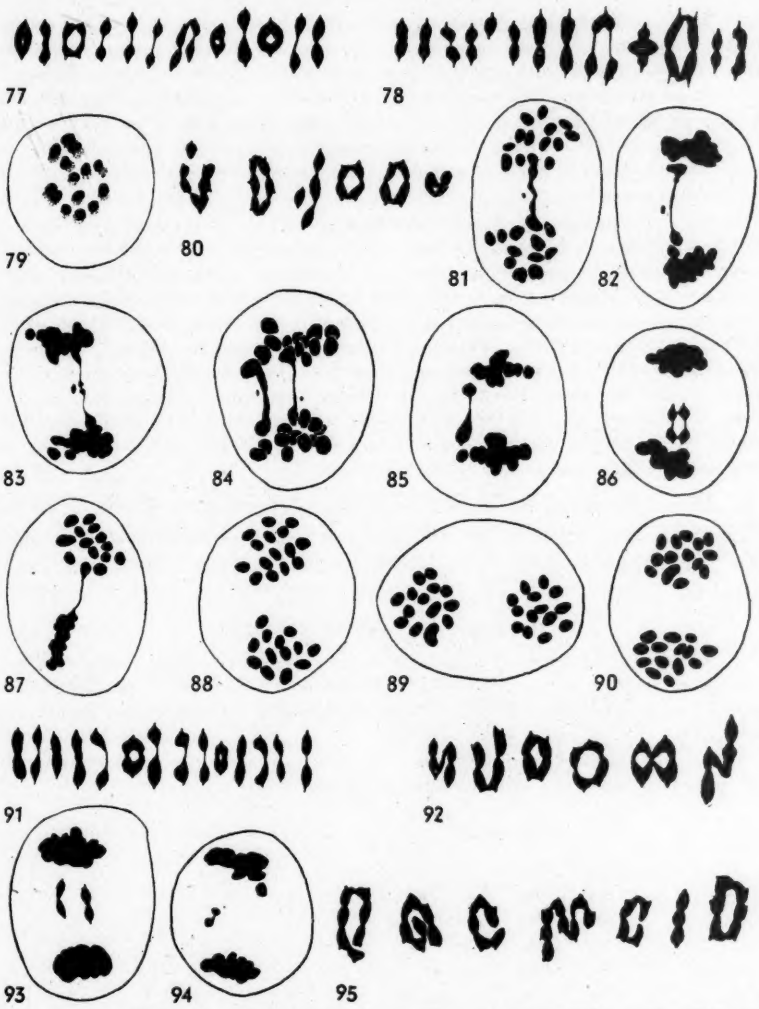
b. *Anaphase-I and later stages.* — In *R 077—5* the anaphases of 145 cells were analysed. The results obtained were that in 124 cells the separation was quite normal, 15 cells showed bridges with fragments (Figs. 81—84) and 6 cells showed delayed separation of different kinds (Figs. 85—86). Thus, about 21 % of the anaphases showed some irregularities.

The bridges with fragments shown in Figs. 81—84 have resulted from a crossing-over between two chromosomes differing in an inverted segment. This segment must have been rather small, as is shown by the size of the fragments and the low frequency of the bridges observed. The two bridges with two fragments observed in one cell (Fig. 84) may be due to the formation of two complementary chiasmata in the same inversion (cf. DARLINGTON, 1937).

The inversion bridges were found to survive to the second division in almost all the cells, as they were observed at metaphase-II in about 12 % of the cells examined (Fig. 87). New bridges in anaphase-II were never observed.

The lagging of chromosomes in the anaphase-I was always of one of two types: the delayed chromosomes formed a bivalent, sometimes with unequal chromosomes (the sex chromosomes?) (Fig. 85), or they were composed of a quadrivalent (Fig. 86). Lagging univalents were never observed in this plant. The delayed quadrivalents were always of the ring-type with normal disjunction shown in Fig. 85.

The chromosome distribution at anaphase-I and metaphase-II was examined in 43 cells. The following frequencies of distribution were obtained: 14 : 14 in 34 cells, 13 : 15 in 3 cells, and 13 : 2 : 13 (one bivalent between the anaphase groups) in 6 cells. These observations show that although most of the gones will receive 14 chromosomes, a low frequency (about 10 %) of cells



Figs. 77—95. Meiosis in *R. tenuifolius*. — Figs. 77—90, *R 077—5*; Figs. 77—78, metaphase-I configurations, drawn separately; Fig. 79, metaphase-I, polar view; Fig. 80, associations of three, four, five and six chromosomes from different metaphase-I groups; Figs. 81—84, anaphase-I with bridges and fragments; Fig. 85, a »lagging» bivalent; Fig. 86, a »lagging» quadrivalent; Fig. 87, a persistent bridge at metaphase-II; Figs. 88—90, metaphase-II groups; Figs. 91—92, *R 0108—4*; Fig. 91, a metaphase-I plate with only bivalents, drawn separately; Fig. 92, some associations from different cells; Figs. 93—94, *R 0109—4*; Fig. 93, two »lagging» bivalents; Fig. 94, a dividing univalent; Fig. 95, *R 0110—42*, associations of three, four, five, six, seven and eight chromosomes from different cells. — Magnification × 2100.

with 13 or 15 chromosomes will be met with. This fact is of special interest when connected with the constancy in chromosome number found within the species, as well as with the good fertility of the pollen grains of this individual.

Some anaphase-I and metaphase-II groups are reproduced in Figs. 88—90. In Fig. 88 the distribution is 13 : 15, but the other figures show the distribution 14 : 14. Secondary pairing is found between some of the chromosomes in Figs. 88—90, but it was more evident in some other cells not reproduced here. Secondary association is often observed in secondary polyploids, as, e. g., the Pomoideae subfamily of Rosaceae (MOFFETT, 1931, 1934; DARLINGTON and MOFFETT, 1930), *Dahlia* (LAWRENCE, 1931), and others, but it is also reported from a number of polyploids, where it may often be an indication of autopolyploidy (cf. DARLINGTON, 1937). Some of the most evident cases of secondary association in a simple polyploid are reported from *Prunus* (DARLINGTON, 1928, 1930; MEURMAN, 1929 b), *Dahlia variabilis* (LAWRENCE, 1931), *Euphorbia* (HAGERUP, 1931), *Dactylis glomerata* (RANCKEN, 1934), and others. In *R. tenuifolius* it may be caused partly by the delayed separation of quadrivalents met with in some cells (cf. Fig. 86), but mainly it is supposed to be of the real type found in the plants mentioned above and only resulting from the affinity of the chromosomes not paired in the prophase.

From the above observations it is evident that *R 077—5 is at the same time heterozygous for an inversion and a segmental interchange.* The significance of this fact for the pollen fertility will be discussed later on.

B. R 084—42, FROM UNDROM.

A rather broad-leaved type with long, repent stems (var. *angustifolius*). Its pollen fertility is not decreased. The meiotic behaviour of this individual proved to be somewhat different from the plant described above, as may be seen from the following observations.

a. *Metaphase-I configurations.* — At metaphase-I the configurations of 20 cells were analysed in side view, giving the following results:

Configuration	Number of cells
$3_{IV} + 8_{II}$	1
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	2
$2_{IV} + 10_{II}$	3
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	2
$1_{IV} + 12_{II}$	5
$1_{III} + 12_{II} + 1_{I}$	4
14_{II}	3
	Total: 20 cells

In 8 of the 20 cells analysed $1_{III} + 1_{I}$ are found, i. e. in 40 % of the cells. This configuration is not composed of the sex chromosomes. Only bivalents are met with in 3 cells, and more than one multivalent in 8 cells. The frequency of multivalents is thus higher than in *R 077—5*, and the highest number of quadrivalents per cell, i. e. $3_{IV} + 8_{II}$, is found in one cell. No

association of more than four chromosomes was observed, and this plant is therefore assumed to be free from heterozygosity for segmental interchange.

The sex chromosomes were found as two bivalents in 10 cells, i. e. in 50 % of the cells studied.

In one cell at metaphase-I one small bivalent was found in an accessory plate between the pole and the normal plate.

b. *Anaphase-I and later stages.* — The distribution of chromosomes was studied in 12 cells only. All these cells, however, showed the distribution 14 : 14. In side views of late anaphase-I 100 cells were examined, 97 of which were quite normal, while 1 cell showed a bridge with a small fragment and 2 cells showed »laggings». In one of these cells the »laggings» were two small chromosomes in the neighbourhood of the poles, and one long bridge-like pair in the middle of the cell, but in the other cell the »laggings» were two bridge-like pairs and some small undefined strings.

In the second division no irregularities were met with, and the distribution of chromosomes in 8 A-II cells was 14 : 14 : 14 : 14.

Plant *R 084—42* seems to be free from heterozygosity for segmental interchange, but it shows a low frequency (1 %) of anaphase-bridges with fragments, indicating a heterozygosity for a small inversion. It also shows a high frequency of univalents and trivalents, which may possibly be the result of heterozygosity for some other differences between the chromosomes.

C. R 0108—4, FROM UNDROM.

A sister individual to the above-mentioned plant.

a. *Metaphase-I configurations.* — The results of an examination of 20 metaphase-I groups are given in the following table:

Configuration	Number of cells
$3_{IV} + 8_{II}$	1
$2_{IV} + 10_{II}$	2
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	1
$1_{IV} + 12_{II}$	8
$1_{III} + 12_{II} + 1_{I}$	2
14_{II}	6
	Total: 20 cells

In this plant quadrivalents are met with in only 60 % of the cells and more than one multivalent are found in 20 % of the cells studied. The highest number of configurations expected per cell, i. e. $3_{IV} + 8_{II}$, is observed in one cell, but no associations of more than four chromosomes are met with in the slides examined. Consequently it must be concluded that *R 0108—4* is not heterozygous for segmental interchange. A metaphase-I plate with only bivalents is shown in Fig. 91, and some quadrivalents from different cells are depicted in Fig. 92.

b. *Anaphase-I and metaphase-II.* — The separation of the two anaphase-I groups was found to be fully normal in all the 200 cells studied. The distribution of chromosomes was always 14 : 14. In metaphase-II secondary association of two and two chromosomes was observed in a great number of cells.

From the above observations it is evident that this plant may be regarded as *free from heterozygosity for segmental interchange and possibly also for inversions.* As, however, plant *R 084—42* from the same sample showed only 1 % of bridges with fragments, it is not entirely out of the question that plant *R 0108—4* is likewise heterozygous for a small inversion, detectable only if much more material was investigated.

D. R 0109—4, FROM RICKOMBERGA.

This plant, which belongs to the first sample of this species obtained by the writer, originates from Rickomberga, in the province of Uppland. It is of the same type as *R 077—5*, i. e. it belongs to var. *minimus.*

a. *Metaphase-I configurations.* — The configurations of 20 metaphase-I plates studied in side view are given in the table below:

Configuration	Number of cells
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	2
$2_{IV} + 10_{II}$	11
$1_{III} + 12_{II} + 1_{I}$	6
14_{II}	1
Total:	20 cells

This plant shows a high frequency of trivalents with univalents, and the configuration $3_{IV} + 8_{II}$ is never observed. The high frequency of trivalents must be due to some lack of homology between these chromosomes. The lack of the configuration $3_{IV} + 8_{II}$ is possibly correlated with the fact that in the somatic divisions only 10 or 11 *v* type autosomes could be observed. The quadrivalents were mostly of the ring type, but in about 20 % of the cells chain-quadrivalents were found.

The sex chromosomes were found as two bivalents in ten cells.

b. *Anaphase-I.* — The distribution of the chromosomes in the anaphase-I plates could not be studied closely. The side views of 136 groups were examined. Of these 125 showed no disturbances, 9 showed a lagging univalent, in one cell a bridge caused by a delayed separation of a quadrivalent was observed, and one cell showed two lagging bivalents (Fig. 93). The univalent divided in 5 cells (Fig. 94), but in the other 4 it goes undivided to one of the poles.

The meiosis of *R 0109—4* is characterized by a rather high frequency of univalents and trivalents at metaphase-I and some low frequ-

ency of lagging univalents at anaphase-I. It is apparently not heterozygous for inversions nor for segmental interchange.

E. R 0110—42, FROM ÖLAND.

An individual of the *R. tenuifolius* var. *minimus* type from the Alvar of the isle of Öland.

a. *Metaphase-I configurations.* — In side views of the metaphase-I of 20 cells the following configurations were observed:

Configuration	Number of cells
$1_{VIII} + 1_{IV} + 8_{II}$	1
$1_{VII} + 1_{IV} + 1_{III} + 7_{II}$	1
$1_{VI} + 1_{IV} + 9_{II}$	4
$1_{V} + 1_{III} + 10_{II}$	1
$2_{IV} + 10_{II}$	2
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	3
$1_{IV} + 12_{II}$	3
$1_{III} + 12_{II} + 1_{I}$	2
14_{II}	3
	Total: 20 cells

In 7 cells, i. e. 35 % of the cells studied, an association of more than four chromosomes was observed, a fact *indicating heterozygosity for a segmental interchange.* Some of the associations from different cells are shown separately in Fig. 95.

The associations of the sex chromosomes will be discussed later on, but it should be mentioned here that in the three cells with the configuration $1_{IV} + 1_{III} + 10_{II} + 1_{I}$ the trivalent and the univalent were composed of *YXX* and *X*.

b. *Anaphase-I and later stages.* — 200 anaphase-I groups showed no disturbances in side view, and in 100 anaphase-II groups no bridges or laggings were observed. The distribution of chromosomes at anaphase-I was studied in 8 cells only, 7 of which showed the distribution 14 : 14, and 1 cell with 13 : 15 chromosomes.

Plant *R 0110—42* is to be regarded as heterozygous for segmental interchange, but it is apparently free from heterozygosity for inversions.

F. R 0111—42, FROM ÖLAND.

This individual is from the same sample as the last-mentioned, but it showed some rather interesting differences from that plant.

a. *Metaphase-I configurations.* — In 20 cells the following configurations were observed in side view:

Configuration	Number of cells
$3_{IV} + 8_{II}$	1
$2_{IV} + 10_{II}$	6
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	2
$1_{IV} + 12_{II}$	6
$2_{III} + 10_{II} + 2_{I}$	1
$1_{III} + 12_{II} + 1_{I}$	1
14_{II}	3
	Total: 20 cells

This individual showed no association of more than 4 chromosomes, but the frequency of quadrivalents is somewhat higher than in the brother plant. Possibly this may be due to the fact that the segmental interchange found in *R 0110—42* includes some of the *v* chromosomes. The trivalents and univalents met with in these two plants are sometimes composed of the sex chromosomes, but some lack of homology must exist in some of the autosomes, too, as trivalent and univalent autosomes are also met with.

b. *Anaphase-I and later stages.* — At late anaphase-I 100 cells were studied in side view. Of these 93 were quite regular, in 5 cells a bridge with a small fragment was detected, and 2 cells showed »laggings», in the first case only one lagging chromosome, in the second case 4 pairs. The distribution 14 : 14 was found in all the 40 cells examined.

Plant *R 0111—42* differed from its brother individual mainly in its being free from segmental interchange and in being heterozygous for an inversion. The distribution of chromosomes at anaphase-I was more regular than in the former plant, owing possibly to the difference in the frequency of multivalents and associations.

G. R 0144—2, FROM OTTSJÖ.

This individual was a carpet-forming, rather broad-leaved plant from Ottsjö, Undersåker, in the province of Jämtland. Morphologically it bore some resemblances to the hexaploid *R. Acetosella* s.str. from the same locality, and therefore it was first supposed to be of a hybridogenous origin. Closer study, however, showed that this was not the case, the plant being only a form with broader leaves than the most common type of the species.

Configuration	Number of cells
$1_{VI} + 11_{II}$	1
$1_{VI} + 1_{III} + 9_{II} + 1_{I}$	1
$1_{VI} + 2_{IV} + 7_{II}$	1
$1_{V} + 1_{IV} + 9_{II} + 1_{I}$	1
$2_{IV} + 10_{II}$	1
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	1
$1_{IV} + 12_{II}$	4
$1_{III} + 12_{II} + 1_{I}$	1
14_{II}	3
	Total: 14 cells

Unfortunately the material of the meiosis of this plant was rather scanty, especially the later stages of the divisions. At metaphase-I 14 plates were studied in side view with the results given on p. 36, at the bottom.

As shown in the table, as many as 4 cells were observed with 1_{VI} or 1_{V}. This indicates that *this plant also is heterozygous for segmental interchange.*

The metaphase-I associations were mostly of the chain type (Fig. 96) and they were almost invariably arranged in a non-zig-zag formation. Thus, other types of distribution than 14 : 14 must be fairly frequent at anaphase-I.

No anaphase-I bridges were observed in this individual.

H. R 0188—42, FROM MALA.

An individual of the form *multifidus*, showing thread-like leaves with divided basal lobes during three different years. It showed the typical repent habit, and its highest stems were about 40 cm.

a. *Metaphase-I configurations.* — In metaphase-I 20 cells were studied in side view with the following results:

Configuration	Number of cells
$3_{IV} + 8_{II}$	2
$3_{IV} + 7_{II} + 2_{I}$	1
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	1
$2_{IV} + 10_{II}$	6
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	1
$1_{IV} + 12_{II}$	7
$1_{IV} + 11_{II} + 2_{I}$	1
14_{II}	1
	Total: 20 cells

No association of more than 4 chromosomes is found in the cells studied, and in none of 30 other cells not completely analysed was any such association observed. Thus this plant is apparently free from heterozygosity for segmental interchange. In two cells two autosomes were found as univalents, and in two other cells the sex chromosomes formed a trivalent and a univalent.

b. *Anaphase-I and later stages.* — The anaphase-I was studied in 100 cells in side view, 90 of which were fully regular, but in 10 cells a bridge with a fragment was observed. *This plant is therefore certainly heterozygous for an inversion.* The distribution of chromosomes was examined in 16 cells, 13 of which showed the distribution 14 : 14, but in three cells the distribution was 15 : 13 chromosomes.

I. R 0604—42, A HYBRID.

This plant originates from a cross between the female plant *R 0110—40* and the male *R 0109—4*, both from Rickomberga. It belonged, of course, to the variety *minimus*, as did both the parents.

a. *Metaphase-I configurations.* — The configurations of 20 cells observed in side view were as follows:

Configuration	Number of cells
$3_{IV} + 8_{II}$	1
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	1
$2_{IV} + 10_{II}$	4
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	3
$1_{IV} + 12_{II}$	2
$2_{III} + 10_{II} + 2_{I}$	1
14_{II}	8
	Total: 20 cells

The configurations met with in the mother individual were not analysed, but the father plant was shown to be free from associations of more than four chromosomes, in 40 % of the cells a trivalent and a univalent were found, and in no cell were 3_{IV} met with. 14_{II} were observed in only 5 % of the cells. The hybrid *R 0604—42* was also free from higher associations, trivalents were observed in only 20 % of the cells, and in two cells more than two multivalents were met with. These minor differences between *R 0109—4* and *R 0604—42* are assumed to be caused by some differences in homology of some of the chromosomes in the population at Rickomberga, giving some variation in metaphase-I configurations, although no heterozygosity for segmental interchange is met with in this population.

b. *Anaphase-I and later stages.* — At anaphase-I some disturbances, such as laggings and delayed separation, were observed in plant *R 0109—4*, but, as no bridges with fragments were observed, this plant was assumed to be free from inversions. Plant *R 0604—42*, however, *proved to be an inversion heterozygote*, as 5 cells with a bridge and a fragment were observed among 100 anaphase-I cells analysed. In 2 cells a lagging univalent was found, but 93 cells were quite regular. The present writer suggests that, although the father plant was free from inversion bridges, it differed from the mother plant in some inverted segment in one of the chromosomes. Such minor differences are assumed to be found in practically all populations of, at any rate, *R. tenuifolius*.

The distribution of chromosomes was examined in 9 cells, 6 of which showed the distribution 14 : 14, but in 3 cells 15 : 13 chromosomes were found.

J. R 0605—42, A HYBRID.

A brother plant to *R 0604—42*.

The metaphase-I configurations were examined in 10 cells only, with the following results:

Configuration	Number of cells
$2_{IV} + 10_{II}$	2
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	1
$1_{IV} + 12_{II}$	3
$1_{III} + 12_{II} + 1_{I}$	1
14_{II}	3
	Total: 10 cells

Univalents were observed in two cells and in three cells 14_{II} were found. No cell showed more than 2_{IV}.

200 cells at late anaphase-I were studied in side view, but none of them showed any disturbances. The plant is, therefore, free from inversions and segmental interchange.

K. R 0609—42, A HYBRID.

A brother plant to the two last-mentioned ones.

The meiosis of this individual showed a number of disturbances, which made a closer analysis of the configurations at metaphase-I almost impossible. At metakinesis and metaphase-I the chromosomes mostly formed a lump which defied close analysis, and it seemed as if the chromosomes themselves were somewhat thicker as is usual in these cells, and possibly they were somewhat »sticky». A closer study of the prophase stages showed that »cytomixis» occurred in nearly 5 % of the cells, and the typical »interchange» of small lumps of chromatin was observed in many cells. Two cells with such an »interchange» are shown in Fig. 97.

At diakinesis—metaphase-I associations which most possibly originate from »cytomixis» were observed in 15 cells out of 80 examined. Fig. 98 shows two cells, the one at metaphase, the other at diakinesis. A bivalent forms a bridge between the cells. Fig. 99 shows another bivalent connecting a cell at metaphase-I to another cell at anaphase-I (?), and Fig. 100 shows two cells connected to each other by a relatively thick bridge between two lumps of chromatin. Fig. 101 shows a group of some cells with some lumps of chromatin of varying size.

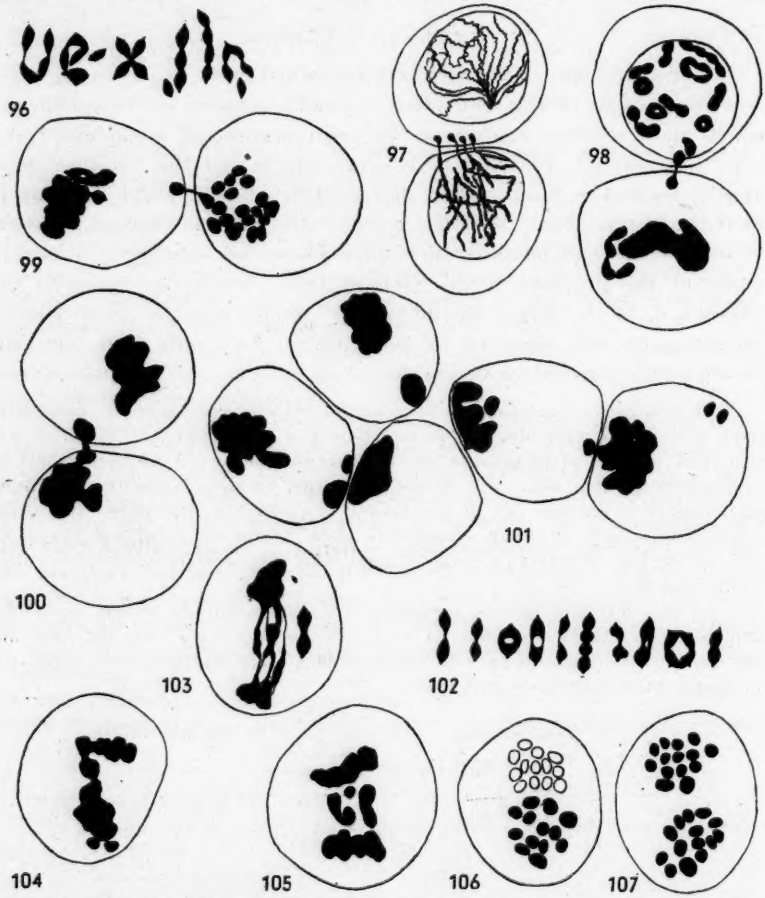
In only 8 cells could the metaphase-I configurations be closely analysed. One of these cells showed only 24 chromosomes ($2_{IV} + 8_{II}$; Fig. 102), but all the others were found to have 28 chromosomes. The configurations observed in these seven cells were as follows:

Configuration	Number of cells
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	2
$2_{IV} + 10_{II}$	2
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	1
$1_{IV} + 12_{II}$	1
14_{II}	1
Total:	7 cells

At anaphase-I the chromosomes seemed to be somewhat sticky, and they seemed to have some difficulty in separating. Some of the formations observed are depicted in Figs. 103—105. At late anaphase-I, however, the separation was apparently the regular one in 135 cells out of 160 examined, 10 cells showed one bridge with a small fragment, indicating heterozygosity for an inversion,

2 cells showed two bridges but only one fragment, 3 cells had a bridge without any detectable fragment, and in 10 cells different kinds of laggings were observed.

As shown by the observation of the cell with 24 chromosomes at metaphase-I, it is obvious that the »cytomictic» disturbances may have some in-



Figs. 96—107. Meiosis in *R. tenuifolius*. — Fig. 96, *R 0144—2*, some multivalents from different cells; Figs. 97—107, *R 0609—42*, with »cytomixis»; Fig. 97, pachytene—diplotene; Fig. 98, diakinesis—metaphase-I; Fig. 99, metaphase-I—anaphase-I; Figs. 100—101, cells with lumps of chromatin of varying size; Fig. 102, metaphase-I with $2n = 24$ chromosomes ($2_{IV} + 8_{II}$), drawn separately; Figs. 103—105, stickiness at anaphase-I; Fig. 106, anaphase-I, 13 : 13 chromosomes; Fig. 107, metaphase-II, 14 : 14 chromosomes. — Magnification × 2100.

fluence on the number of chromosomes in the cells. At anaphase-I and metaphase-II the chromosome number could be counted in both the plates in 40 cells only, but in one of these cells the number was 12 : 12, 3 cells had 13 : 13 chromosomes (Fig. 106), one showed the distribution 13 : 15, but all the other 35 cells had 14 : 14 chromosomes (Fig. 107).

At anaphase-II 45 cells were examined, 41 of which were quite regular, 2 cells showed bridges and in 2 other cells some undefined laggings were observed.

According to the observations made on the prophase—metaphase—anaphase stages, cells with supernumerary chromosomes were to be expected. No such cell was, however, observed at anaphase-I and -II nor at metaphase-I in the cells analysed.

The phenomenon of »cytomixis» (GATES, 1911) will not be discussed in this paper, but a detailed discussion on the subject will be found in the papers by, e. g., CHURCH (1929), KATTERMANN (1932), and VAARAMA (1941). In *R. tenuifolius* it is undoubtedly the result of some disturbances in the pre-meiotic divisions, possibly due to some interruptions in the nucleic acid metabolism of the chromosomes causing stickiness of the mitotic chromosomes. Such stickiness is found to cause some irregularities at mitotic anaphase in plants after cold-treatment (cf. DARLINGTON and LA COUR, 1938, 1942; GEITLER, 1940). This assumption may be somewhat supported by the fact that the fixation of the flowers of *R 0609—42* was made after a period of cold and rainy weather, but it is somewhat contradicted by the fact that »cytomixis» has not yet been reported from material experimentally treated with cold.

L. R 0610—42, A HYBRID.

An individual of the same cross as the three last-mentioned plants.

This plant was very like plant *R 0604—42*, as will be seen upon comparing the metaphase-I configurations of these two plants. The frequency of cells with 14_{II} was, however, somewhat lower in *R 0610—42*. The results of the analysis of 20 cells at metaphase-I are given below:

Configuration	Number of cells
$3_{IV} + 8_{II}$	1
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	1
$2_{IV} + 10_{II}$	7
$1_{IV} + 2_{III} + 8_{II} + 2_{I}$	1
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	3
$1_{IV} + 12_{II}$	4
$1_{III} + 12_{II} + 1_{I}$	1
14_{II}	2
	Total: 20 cells

Only 37 cells were analysed at anaphase-I, and two of them showed a bridge with a fragment. *The plant may thus be regarded as heterozygous for an inversion.*

M. R 0615—42, A HYBRID.

A plant of the same sample as the four last-mentioned ones.

The configurations met with in 20 cells at metaphase-I were as follows:

Configuration	Number of cells
$3_{IV} + 8_{II}$	2
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	5
$2_{IV} + 10_{II}$	3
$1_{IV} + 2_{III} + 8_{II} + 2_{I}$	1
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	4
$1_{IV} + 12_{II}$	3
$1_{III} + 12_{II} + 1_{I}$	1
14_{II}	1
	Total: 20 cells

At anaphase-I 100 cells were examined. Of these, 91 showed regular separation, *6 showed a bridge with a fragment*, and 3 cells showed »laggings» of some undefined kind.

N. R 0804—42, A HYBRID.

This individual was obtained from a cross between a female plant of the variety *minimus* from Rickomberga and a male plant of var. *typicus* from Lomma in the province of Scania. Morphologically the hybrid closely resembled its father plant.

a. *Metaphase-I configurations.* — At metaphase-I one or more univalents were very frequent, indicating a lack of homology in some pairs of chromosomes. The configurations at metaphase-I were analysed in side view in 12 cells, giving the following results:

Configuration	Number of cells
$1_{VIII} + 1_{IV} + 7_{II} + 2_{I}$	1
$1_{VII} + 2_{IV} + 6_{II} + 1_{I}$	1
$2_{VI} + 1_{IV} + 5_{II} + 2_{I}$	2
$2_{VI} + 6_{II} + 4_{I}$	1
$1_{VI} + 3_{IV} + 5_{II}$	1
$1_{VI} + 2_{IV} + 5_{II} + 4_{I}$	2
$1_{VI} + 1_{IV} + 1_{III} + 6_{II} + 3_{I}$	1
$1_{VI} + 1_{IV} + 8_{II} + 2_{I}$	1
$1_{V} + 2_{IV} + 6_{II} + 3_{I}$	1
$2_{IV} + 9_{II} + 2_{I}$	1
	Total: 12 cells

As shown in the table, associations of more than four chromosomes are met with in 11 cells out of the 12 examined. In three cells two associations of six chromosomes are met with, indicating that *this individual is heterozygous for two segmental interchanges.*

b. *Anaphase-I.* — The univalents found at metaphase-I seem to go to the poles together with the other chromosomes, as 38 cells out of 50 examined were fully regular, 7 showed 1—4 lagging univalents (3 cells with one, 2 cells with two, 1 cell with three, and 1 cell with four univalents), 4 cells showed one bridge with a fragment, and in 1 cell two bridges with fragments were observed. The univalents were dividing in 5 cells (2 cells with no dividing univalent, 4 cells with one dividing, and 1 cell with two dividing univalents). The distribution of chromosomes could not be examined in any of the cells.

The meiotic irregularities met with in this hybrid between the two morphologically different forms from Uppland and Scania show that the parents differed in two segmental interchanges and in one inverted segment, in addition to a lack of homology between some pairs of chromosomes.

O. R 0814, A HYBRID.

The individual *R 0814* was obtained from a cross between a female plant from Rickomberga and the male plant *R 0144—5* from Ottsjö. Morphologically it was very like the mother plant, although its leaves were somewhat broader.

a. *Metaphase-I.* — At metaphase-I univalents and multivalents were very frequent. The configurations were completely analysed in three cells only, showing $1_{IX} + 1_{III} + 7_{II} + 2_{I}$; $1_{VI} + 1_{V} + 1_{III} + 5_{II} + 4_{I}$ and $2_{IV} + 3_{III} + 4_{II} + 3_{I}$ respectively. Some multivalents observed in different cells are reproduced in Fig. 108. One cell showed a high degree of asyndesis (Fig. 109) with only 2_{II} and 24_{I}. Outside a great number of metaphase-I plates some univalents were observed (Figs. 110—112).

The two configurations with associations of 9 as well as of 6 and 5 chromosomes in each cell must be due to the individual being *heterozygous for two segmental interchanges between non-homologous chromosomes*, but the asyndesis must be mainly of a physiological nature as it can only partly be caused by a lack of affinity between the chromosomes.

b. *Anaphase-I.* — The number of univalents was counted in 120 cells at anaphase. The following results were obtained:

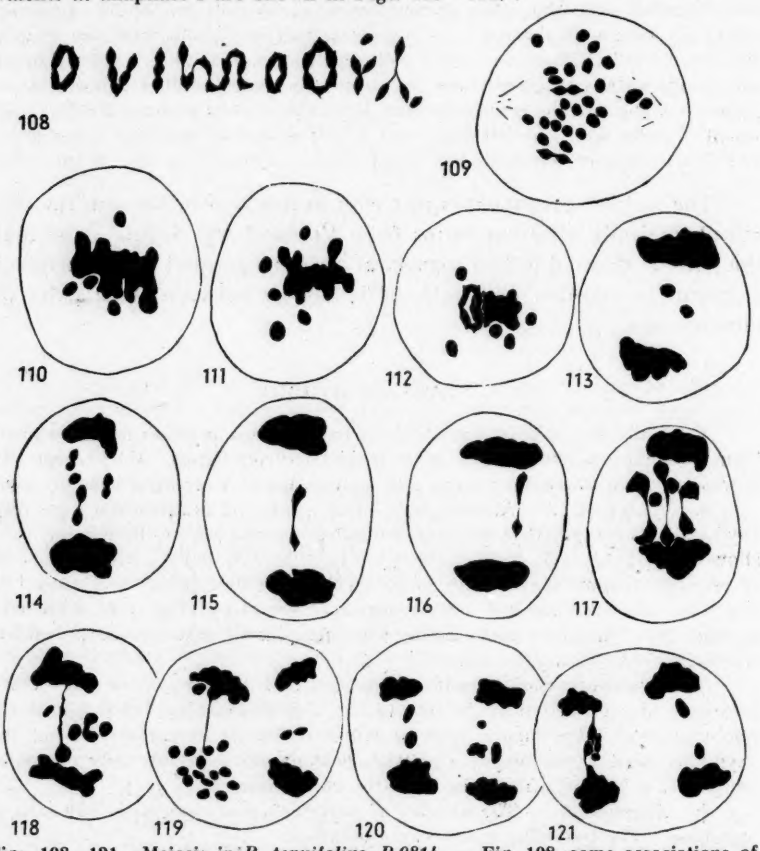
Number of univalents:	*0*	*1*	*2*	*3*	*4*	*5*	*6*
» » cells:	15	30	24	22	15	9	5

These values correspond to 87,5 % of cells with one or more univalents at anaphase-I.

The division of the univalents was studied in all the 120 cells. The values obtained were:

Number of dividing univalents:	*0*	*1*	*2*	*3*	*4*	*5*	*6*
» » cells:	41	47	20	7	4	1	0

These results show that the univalents do not divide in 34 % of the cells only, but in no cell with six univalents do all divide. Some cells with univalents at anaphase-I are shown in Figs. 113—114.



Figs. 108—121. Meiosis in *R. tenuifolius, R 0814*. — Fig. 108, some associations of three, four, five, six and nine chromosomes from different cells; Fig. 109, a cell with high degree of asyndesis ($2_{II} + 24_{I}$); Figs. 110—112, metaphase-I with univalents outside the plate; Figs. 113—114, anaphase-I with »lagging» univalents; Figs. 115—117, anaphase-I with »lagging» bivalents; Fig. 118, a bridge with a fragment and »lagging» univalents at anaphase-I; Figs. 119—121, »laggings», etc. at anaphase-II. — Magnification × 2100.

In some few anaphase-I cells lagging bivalents with unequal chromosomes were observed (Figs. 115—116). It is not impossible that these may be the

XY pair of sex chromosomes, but the unequal pair might also be the result of the heterozygosity for segmental interchanges in the autosomes. In one cell (Fig. 117) a delayed separation of two rings and one rod bivalent was observed.

Evidence of heterozygosity in an inversion was obtained in both anaphase-I and -II. One bridge with a rather large fragment is shown in Fig. 118, and in Fig. 119 a bridge in anaphase-II is seen.

Only a limited number of interphases were found in the material, but lagging chromosomes or micronuclei were fairly frequent and some »restitution»-nuclei were also found.

c. *The second division.* — Only a few cells were closely studied in the second division. The metaphase-II was mostly quite regular; in some cells, however, univalents were seen outside the plate. Almost all the anaphase-II groups showed lagging chromosomes (Fig. 120) or bridges with laggings (Fig. 121). In 9 out of 100 telophase-II cells a »restitution»-nucleus was observed. It might be caused by a bridge with laggings, as found in Fig. 121.

In the tetrad stage most of the about 500 tetrads studied showed four cells with somewhat differently sized nuclei. A low frequency (2 cells observed) of dyads is met with, as well as triads with three cells of different size or with a small extra cell. Pentads with one small nucleus were not infrequent.

To summarize the results from the two last-mentioned hybrids, *R 0804—42* and *R 0814*, it must be emphasized that *they are both the results of crosses between different varieties from different localities.* Plant *R 0804—42* is a hybrid between a sister plant to *R 0109—4* from the population from Rickomberga, with a high frequency of trivalents and univalents and a low frequency of anaphase-bridges, and a male plant, not cytologically studied, from Lomma. Plant *R 0814* was a hybrid between a female plant from Rickomberga and the male plant *R 0144—42*, which was found to be heterozygous for segmental interchange. If the karyotype differences in *R.* sg. *Acetosella* are always due to segmental interchange, the plants with associations of more than four chromosomes must be regarded as hybrids between two symmetrical karyotypes. Moreover, the lack of pairing can be regarded as an indication of hybridity between two or more karyotypes. *The meiotic irregularities met with in the two hybrids R 0804—42 and R 0814 may then be explained as being due to hybridity between at least three or four different karyotypes.* Thus the three different varieties used as parents in the two crosses must differ in at least two or three segmental interchanges.

P. R 0829—42, FROM BUDAPEST.

The material from Budapest consisted of plants with long thin leaves of the common *tenuifolius* type. The height of the plants was between 40 and 50 cm. and their habit erect.

a. *Metaphase-I.* — The metaphase-I plates were often found at one side of the cell or half way between the one pole and the central part of the cell. Consequently the spindle was sometimes asymmetrical or not of the same length on both sides of the plate.

The metaphase-I configurations were analysed in 20 cells in side view with the following results:

Configuration	Number of cells
$1_{VI} + 1_{IV} + 9_{II}$	1
$1_{VI} + 1_{III} + 9_{II} + 1_{I}$	1
$1_{V} + 2_{IV} + 7_{II} + 1_{I}$	1
$3_{IV} + 8_{II}$	2
$2_{IV} + 2_{III} + 7_{II}$	1
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	2
$2_{IV} + 10_{II}$	6
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	2
$1_{IV} + 12_{II}$	3
14_{II}	1
	Total: 20 cells

In three cells associations of more than four chromosomes were observed, indicating that *this individual is heterozygous for segmental interchange.* In six cells one trivalent and one univalent were observed, which must be due to a lack of homology between the chromosomes of a »quadrivalent». In one cell two trivalents without univalents were found. They are possibly composed of the same chromosomes as the associations of six chromosomes in the two other cells. The association of six formed a ring in one of the cells, but it was found as a chain in the other. The sex chromosomes were found as bivalents in all the cells examined.

b. *Anaphase-I and later stages.* — At anaphase-I 100 cells were examined, but no irregularities were observed. Nor were any defects found in 200 cells at the second division.

Q. R 0831—42, FROM CLUJ.

The sample from Cluj, Roumania, belonged to the rather broad-leaved type, var. *angustifolius.*

a. *Metaphase-I configurations.* — The configurations at metaphase-I were analysed in 20 cells with the results given on p. 47, at the top.

As shown in the table, associations of more than four chromosomes were found in 7 cells, i. e. in 35 % of the cells analysed. *Plant R 0831—42 is therefore heterozygous for a segmental interchange.* In ten cells two univalents were found, likewise an indication of the heterozygosity of the individual. The sex chromosomes were found as two bivalents in 19 out of the 20 cells studied.

b. *Anaphase-I and later stages.* — At late anaphase-I 100 cells were examined, 86 of which were quite regular. In 7 cells one bridge with a fragment was found, 1 cell showed two bridges with fragments, 4 cells showed two lagging univalents, and in 2 cells one lagging univalent was observed.

Configuration	Number of cells
$1_{VIII} + 1_{IV} + 8_{II}$	1
$1_{VII} + 10_{II} + 1_{I}$	1
$1_{VI} + 2_{IV} + 7_{II}$	2
$1_{VI} + 1_{IV} + 9_{II}$	1
$1_{VI} + 1_{IV} + 8_{II} + 2_{I}$	2
$3_{IV} + 8_{II}$	2
$3_{IV} + 7_{II} + 2_{I}$	3
$2_{IV} + 10_{II}$	1
$2_{IV} + 9_{II} + 2_{I}$	2
$1_{IV} + 12_{II}$	1
$1_{IV} + 11_{II} + 2_{I}$	3
14_{II}	1
	Total: 20 cells

Thus, *the plant is also heterozygous for an inversion.* The laggings were found as micronuclei in 8 out of 30 interphases.

The distribution of chromosomes was examined in 25 cells at anaphase-I and metaphase-II. 18 cells showed the distribution 14 : 14, but in 7 cells there were 13 : 15 chromosomes.

At anaphase-II 80 cells were studied. 69 cells showed quite regular separation, 5 showed a bridge, and in 6 cells some laggings were observed.

Some microcells were observed in a low frequency at the tetrad stage.

R. R 0834—42, FROM CLUJ.

This individual is a brother plant to the last-mentioned one.

The metaphase-I configurations observed in the cells analysed were as follows:

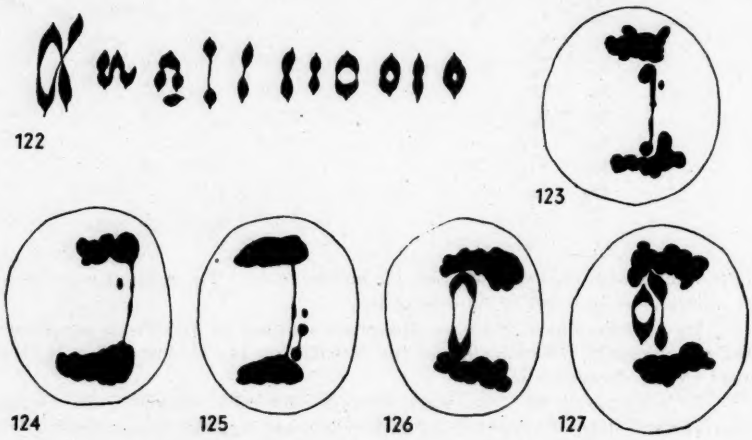
Configuration	Number of cells
$3_{IV} + 1_{III} + 6_{II} + 1_{I}$	1
$3_{IV} + 8_{II}$	2
$2_{IV} + 10_{II}$	4
$2_{IV} + 9_{II} + 2_{I}$	1
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	4
$1_{IV} + 12_{II}$	3
$1_{IV} + 11_{II} + 2_{I}$	1
$1_{III} + 12_{II} + 1_{I}$	1
14_{II}	2
$13_{II} + 2_{I}$	1
	Total: 20 cells

This individual is not heterozygous for segmental interchange, and its frequency of univalents is somewhat lower than that found in its brother plant.

At anaphase-I 100 cells were examined, 97 of which were regular, but 3 cells showed a lagging univalent. At anaphase-II one »restitution»-nucleus

was observed in one cell, and in one cell at metaphase-II one plate was found with 28 chromosomes.

This plant differed from its brother individual in being apparently free from heterozygosity for segmental interchange and inversions.



Figs. 122—127. Meiosis in *R. tenuifolius, R 0836.* — Fig. 122, metaphase-I chromosomes, drawn separately; Figs. 123—124, bridges with fragments at anaphase-I; Fig. 125, a bridge, a fragment and a dividing univalent at anaphase-I; Figs. 126—127, delayed separation of bivalents at anaphase-I. — Magnification × 2100.

S. R 0836, FROM LIÈGE.

R 0836 was obtained from seeds originating from the neighbourhood of Liège in Belgium. It was about 25 cm. high with typical leaves and habit, and it flowered some weeks after sowing and died the next winter.

The metaphase-I configurations were closely examined in only a very few cells. One such plate is drawn separately in Fig. 122. It shows the configuration $2_{IV} + 1_{III} + 8_{II} + 1_{I}$, but in three other cells the configuration $2_{IV} + 10_{II}$ was observed. In some cells (7) a univalent goes to one of the poles, while the other chromosomes are still at metaphase-I.

The anaphase-I was examined in 126 cells. 100 showed the regular separation, 14 showed bridges with fragments (Figs. 123—125) and 12 showed a delayed separation of bivalents (Figs. 126—127) or »lagging» univalents.

The distribution of chromosomes at anaphase-I could not be studied in the material, but in 20 metaphase-II cells 14 chromosomes were counted in at least one of the plates.

Most of the tetrads studied were normal, but tetrads with two cells degenerating as well as with pentads and monads were observed in some low frequency.

T. R 0844—42, FROM MOSCOW.

The material from Moscow belonged to a rather broad-leaved type of the variety *angustifolius* as well as of the main type of var. *typicus*. This plant was of the var. *angustifolius* type.

The metaphase-I configurations were analysed in 20 cells with the following results:

Configuration	Number of cells
$3_{IV} + 8_{II}$	3
$2_{IV} + 1_{III} + 8_{II} + 1_{I}$	3
$2_{IV} + 10_{II}$	2
$2_{IV} + 9_{II} + 2_{I}$	1
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	1
$1_{IV} + 12_{II}$	5
$1_{IV} + 11_{II} + 2_{I}$	1
14_{II}	4
	Total: 20 cells

The plant is apparently free from associations of more than four chromosomes, but univalents are found in 6 cells, 2 of which are free from trivalents.

At anaphase-I no irregularities were met with in the 100 cells examined, while 200 cells at anaphase-II showed quite regular separation.

U. R 0847—42, FROM MOSCOW.

This individual from Moscow belonged to the var. *typicus* with thread-like leaves and repent mode of growth.

a. *Metaphase-I configurations.* — The configurations at metaphase-I were examined in 20 cells with the following results:

Configuration	Number of cells
$1_{VIII} + 1_{VI} + 7_{II}$	1
$1_{VIII} + 2_{IV} + 6_{II}$	1
$1_{VII} + 1_{VI} + 1_{IV} + 5_{II} + 1_{I}$	1
$2_{VI} + 1_{IV} + 6_{II}$	2
$1_{VI} + 1_{V} + 1_{IV} + 6_{II} + 1_{I}$	1
$1_{VI} + 2_{IV} + 7_{II}$	1
$1_{VI} + 1_{IV} + 9_{II}$	1
$1_{VI} + 1_{IV} + 8_{II} + 2_{I}$	1
$1_{V} + 2_{IV} + 7_{II} + 1_{I}$	1
$3_{IV} + 8_{II}$	2
$2_{IV} + 10_{II}$	3
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	2
$1_{IV} + 12_{II}$	2
$2_{III} + 10_{II} + 2_{I}$	1
	Total: 20 cells

In 10 cells out of the 20 cells studied, associations of more than four chromosomes are found, and in 5 cells two such associations ($1_{VIII} + 1_{VI}$; $1_{VII} + 1_{VI}$; 2_{VI}; $1_{VI} + 1_{V}$) are observed. *This must be due to heterozygosity for two segmental interchanges* in different groups of chromosomes, as no association of more than eight chromosomes is found.

b. *Anaphase-I.* — At anaphase-I no disturbances were met with in 100 cells examined, while 100 anaphase-II cells were quite regular. Unfortunately, countings could only be made in 20 cells in order to study the distribution of chromosomes at anaphase-I. Of these cells 7 showed the distribution 13 : 15, in 2 cells the distribution was 12 : 16, and in 11 cells the distribution was the regular one, 14 : 14.

V. SUMMING UP.

The results from the examinations of the meiotic behaviour of the 21 plants of *R. tenuifolius* may be summarized as follows:

All the individuals are found to have a relatively low frequency of multivalents owing to the presumed autopolyploidy of the species. Meiotic irregularities of different kinds are fairly frequent. The most frequent disturbances are trivalents with univalents at metaphase-I observed in 20 plants, and in 11 plants two or more univalents are met with, owing to some lack of homology between the sets of chromosomes. 11 plants (about 52 % of the material studied) were found to be heterozygous for an inversion, and evidence of heterozygosity for segmental interchange was obtained in 8 plants (about 33 % of the material studied), 3 of which were heterozygous for two segmental interchanges.

3. MEIOSIS IN RUMEX ACETOSELLA S. STR.

Previous investigations. — The meiosis of this hexaploid species has been somewhat extensively examined by MEURMAN (1925 a, b), KIHARA (1925, 1927 a), and JENSEN (1936 a, b). Unfortunately, these authors made no close study of the behaviour of all the chromosomes in a number of cells from the same individual, as they mainly studied the supposed multipartite complex of sex chromosomes. Some of the descriptions and illustrations made by these authors are, however, of great interest, as they indicate the occurrence of multivalents and univalents at metaphase-I and bridges and delayed separation of bivalents and multivalents at anaphase-I, as the present writer has also found in the above-mentioned tetraploid species.

MEURMAN (1925 b) seems to have worked mainly with a male plant with $2n = 41$ chromosomes. Judging from his illustrations and descriptions at least one trivalent — according to him the sex chromosomes — is found in each cell, but in, for instance, his Fig. 35 it is not

out of the question that, in addition, a hexavalent is to be found in polar view, although it might also be interpreted as a secondary association of three bivalents. At anaphase-I MEURMAN observed »an elongated chromosome», which in some of the illustrations may be due to delayed separation of multivalents or bivalents, but most frequently it seems to be an inversion bridge.

KIHARA (1925) worked with males with $2n = 41$ and 42 chromosomes. According to his drawings one quadrivalent or one hexavalent is found in almost every cell at metaphase-I. In the plant with $2n = 41$ chromosomes lagging univalents and bivalents are drawn at anaphase-I. The paper by KIHARA (1925) is in Japanese with only an explanation of the figures in English, but later on (1927 a) he published a more extensive paper on his results in German.

In the last-mentioned paper KIHARA closely described the meiotic behaviour of the multivalents met with in some male individuals from Germany and Denmark. He found that at least two groups of three chromosome pairs (aa, bb, cc and ll, mm, nn) were able to form hexavalents or quadrivalents, and in addition to this he noted an octovalent in one individual. The configurations found by him to be characteristic of the 15 plants studied are listed in the table below:

Configuration	Number of plants
Plants with $2n = 42$ chromosomes	
$1_{VIII} + 17_{II}$	1
$1_{VI} + 3_{IV} + 12_{II}$	1
$1_{VI} + 1_{IV} + 1_{III} + 14_{II} + 1_{I}$	1
$1_{VI} + 1_{IV} + 16_{II}$	2
$1_{VI} + 18_{II}$	2
$1_{IV} + 19_{II}$	2
$1_{IV} + 18_{II} + 2_{I}$	1
21_{II}	1
Total: 11 plants with $2n = 42$	
Plants with $2n = 41$ chromosomes	
$1_{VI} + 17_{II} + 1_{I}$	1
$1_{IV} + 18_{II} + 1_{I}$	1
$20_{II} + 1_{I}$	1
Total: 3 plants with $2n = 41$	
Plant with $2n = 43$ chromosomes	
$1_{VI} + 1_{IV} + 16_{II} + 1_{I}$	1

Unfortunately, KIHARA gives no account of the number of cells with the different configurations, nor does he seem to have made very extensive studies of the variation in the number of multivalents in different cells of the same individual. His results indicate, however, that different individuals show great variability in forming multivalents, as might be expected from the studies of the tetraploid species made by the present writer.

As shown in the table, KIHARA reported the configuration 21_{II} from one plant with $2n = 42$ chromosomes and $20_{II} + 1_{I}$ from one plant with $2n = 41$, all the other plants showing one to four multivalents. The individual with $1_{VIII} + 17_{II}$ must be regarded as heterozygous for a segmental interchange. These facts are in full accord with the results obtained by the present writer from the tetraploid as well as the hexaploid species. For my part, however, I question whether all the cells of the two individuals reported to be free from multivalents really showed only bivalents in all the cells.

KIHARA (1927 a) made excellent studies of the prophase stages of the hexaploid species, although some of his results seem to be somewhat doubtful in the light of modern cytology. The present writer, however, has not yet succeeded in an attempt to analyse the prophase stages of any of the species studied, although the same methods were used as those described by KIHARA.

The latest contributions to the studies of meiosis in *R. Acetosella* s.str. are given by JENSEN (1936 a, b), likewise without any close examination of the frequency of the configurations at metaphase-I or the frequency of different irregularities at anaphase-I. He states (1936 a), however, that »lateral views of the heterotypic metaphase of microsporogenesis clearly show to what extent the chromosomes lag on the spindle. The early anaphase is also abnormal in that a varying number of strings and laggards are present.» In the later work (1936 b) he states that »the heterotypic metaphases are so irregular that one can hardly determine what occurs most often.» He could not for the most part analyse the multivalent complexes at metaphase-I and in some cases he apparently failed to find any multivalents at all in the plates. In his illustrations, however, we find some multivalents of undefined structure in all the three cells drawn, and univalents in two of the cells. At anaphase-I JENSEN (1936 b) found both a lagging of chromosomes and bridges, and his Fig. 20 shows two bridges which may possibly have been caused by crossing-over in an inversion. He also tried to make some counts of the distribution

of chromosomes at anaphase-I. The results of his efforts was that »it appeared that there was a variation from 17 to 23 in the groups counted». No exact counts seem to have been made and he concludes (1936 a) that the haploid number in *R. Acetosella* s.str. is n = 22, but he reproduced only 21 chromosomes at anaphase-I in the later work (1936 b).

JENSEN (1936 a, b, 1939, 1940) concluded from his results that the hexaploid species *R. Acetosella* s.str. must be of a hybridogenous origin as he found some of the same irregularities in hybrids between some other species of *Rumex* as well as in some other hybrids. The present writer suggests, however, that all the irregularities found by JENSEN, KIHARA, and MEURMAN in this species must be mainly due to its being an autopolyploid in the wide sense of the word, and that almost every individual of the species may be composed of more than one different karyotype. In the strict sense, however, every individual of this allogamous hexaploid species is a hybrid, but not in the same sense as that proposed by JENSEN.

Own results. — The present writer has attempted to make a closer analysis of the meiotic behaviour of his material of this hexaploid species. As, however, it is extremely difficult completely to analyse all the configurations found at metaphase-I, he did not succeed in analysing the meiosis in more than 15 male individuals. Nevertheless, he has been able to establish that all but one of the individuals examined seem to be heterozygous for one or more inversions, and different kinds of laggings are frequently met with at anaphase-I. As shown below, wide variation is found in the formation of multivalents, as KIHARA (1927 a) also found, and associations of more than six chromosomes, indicating a heterozygosity for segmental interchange, have been observed in some of the plants examined.

A. R 0133—39, FROM FRÖSÖN.

This plant belongs to a sample of plants from Frösön in the province of Jämtland. It is a type with normal broad leaves and a somewhat repent mode of growth, such as is quite commonly found in the material of the hexaploid species from these regions. Its chromosome number was $2n \doteq 43$ and its vegetative vigour is normal.

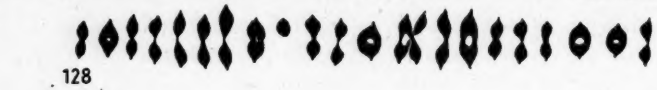
a. *Metaphase-I configurations.* — The metaphase-I configurations were analysed in 14 cells with the results given on p. 54, at the top.

In no cell was more than one multivalent found, and hexavalents were never observed. The extra chromosome was mostly found as a univalent, but in one cell it seems to have paired and formed a trivalent together with a bivalent, or a bivalent together with one chromosome from the quadrivalent

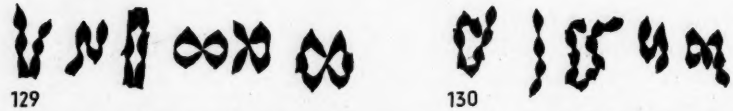
Configuration	Number of cells
$1_{IV} + 19_{II} + 1_{I}$	6
$1_{III} + 19_{II} + 2_{I}$	2
$1_{III} + 20_{II}$	1
$21_{II} + 1_{I}$	4
$20_{II} + 3_{I}$	1
	Total: 14 cells

found in some of the other cells. The chromosomes of a cell with $1_{IV} + 19_{II} + 1_{I}$ drawn separately are depicted in Fig. 128.

b. *Anaphase-I and later stages.* — At anaphase-I 100 cells were examined in side view. 9 cells showed a bridge with a fragment, in 2 cells two bridges with fragments were met with, 4 cells showed one or two lagging univalents,



128



129 130

Figs. 128—130. Meiosis in *R. Acetosella* s.str. — Fig. 128, metaphase-I chromosomes of one cell in *R 0133—39*, drawn separately; Fig. 129, some multivalents from different cells in *R 0135—39*; Fig. 130, associations of four, six, eight and ten chromosomes from *R 0138—39*. — Magnification × 2100.

but 85 cells were quite regular. The cells with bridges and fragments indicate the occurrence of *heterozygosity for one inversion.*

The distribution of chromosomes at anaphase-I was studied in 10 cells, 8 of which showed the distribution 21 : 22, but in two cells the distribution was 20 : 23 in the plates.

B. R 0135—39, FROM FRÖSÖN.

A plant from the same population as the last-mentioned plant. Its chromosome number was $2n = 42$.

Configuration	Number of cells
$2_{VI} + 3_{IV} + 9_{II}$	3
$2_{VI} + 2_{IV} + 11_{II}$	2
$2_{VI} + 1_{IV} + 13_{II}$	1
$1_{VI} + 1_{V} + 3_{IV} + 9_{II} + 1_{I}$	1
$1_{VI} + 3_{IV} + 11_{II} + 2_{I}$	1
$1_{VI} + 2_{IV} + 14_{II}$	4
$1_{VI} + 18_{II}$	1
$3_{IV} + 15_{II}$	1
	Total: 14 cells

a. *Metaphase-I configurations.* — In 14 cells the metaphase-I configurations given on p. 54, at the bottom, were observed.

Multivalents are found in all the cells, and the highest configuration observed, $2_{VI} + 3_{IV} + 9_{II}$, is met with in 3 cells. Some multivalents from six different cells are drawn separately in Fig. 129.

b. *Anaphase-I and later stages.* — The individual was found to be *heterozygous for an inversion* as 10 out of 100 cells examined showed a bridge with a small fragment at anaphase-I. The distribution of chromosomes at anaphase-I was studied in 11 cells. 7 cells showed the distribution 21 : 21, 2 cells had 20 : 22 chromosomes, and in 2 cells the distribution was found to be 19 : 23. Secondary association of two and three chromosomes was observed in some cells at metaphase-II.

C. R 0138—39, AN INTERSEXUAL PLANT FROM FRÖSÖN.

This individual from Frösön was vegetatively very vigorous, with the typical broad leaves like the other plants of this population. It bears mostly male flowers, but about 10 % of the latest flowers were found to be hermaphroditic, and after isolation they yielded some germinative seeds. According to ONO (1930), an intersexual plant of *R. Acetosella* s.str. studied by him proved to have only 2n = 41 chromosomes, but this individual had the normal hexaploid chromosome number 2n = 42.

a. *Metaphase-I configurations.* — The configurations at metaphase-I were analysed in 11 cells only, with the following results:

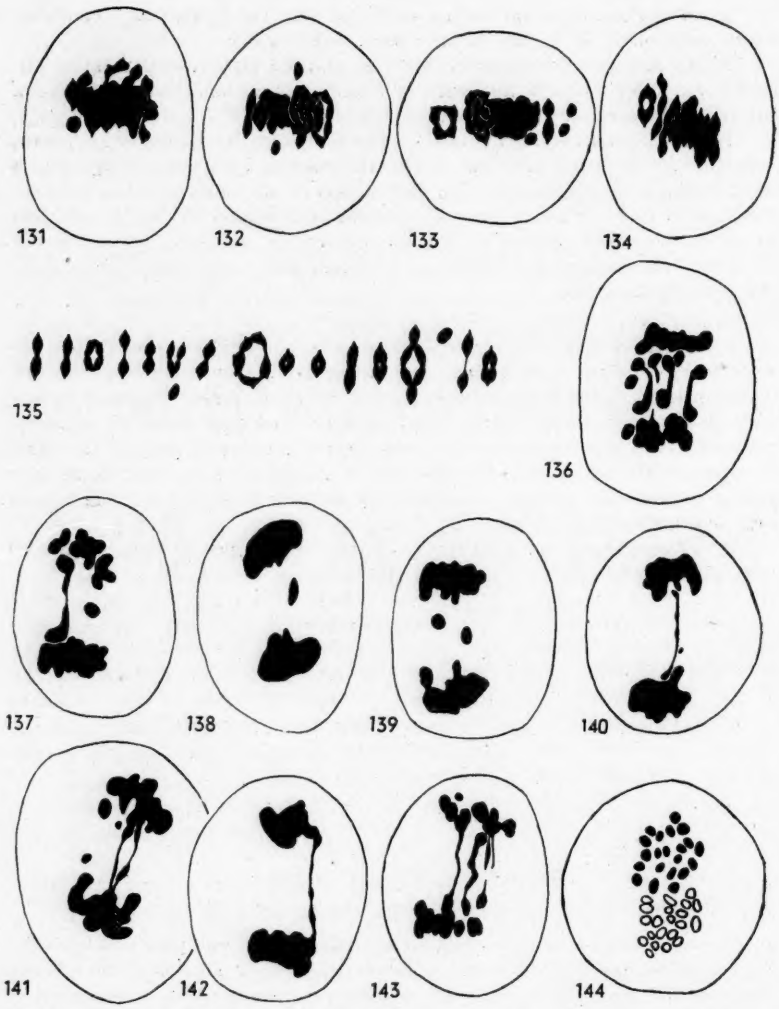
Configuration	Number of cells
$1_X + 2_{IV} + 12_{II}$	1
$1_{VIII} + 1_{VI} + 3_{IV} + 8_{II}$	1
$1_{VII} + 1_V + 1_{IV} + 13_{II}$	1
$1_{VI} + 3_{IV} + 12_{II}$	2
$1_{VI} + 2_{IV} + 14_{II}$	1
$1_{VI} + 1_{IV} + 16_{II}$	1
$3_{IV} + 15_{II}$	1
$3_{IV} + 14_{II} + 2_I$	1
$2_{IV} + 17_{II}$	1
$2_{IV} + 16_{II} + 2_I$	1
	Total: 11 cells

One association of more than six chromosomes is met with in three cells. This indicates that *the individual is heterozygous for a segmental interchange.* Some of the associations met with in different cells are drawn separately in Fig. 130.

b. *Anaphase-I.* — The separation at anaphase-I was found to be regular in 97 cells, but 3 cells showed a bridge with a small fragment. Thus, *this individual is heterozygous both for an inversion and for segmental interchange.*

D. R 0140—39, FROM FRÖSÖN.

A plant belonging to the same population as the three above-mentioned. Its chromosome number was 2n = 42.



Figs. 131—144. Meiosis in *R. Acetosella* s.str., *R 0140—39*. — Fig. 131, metaphase-I group with undefiniable multivalents and one univalent outside the plate; Fig. 132, metaphase-I group with one hexavalent ring and two univalents; Fig. 133, metaphase-I group with a quadrivalent ring and a heptavalent chain and three univalents; Fig. 134, metaphase-I group with a hexavalent and a trivalent; Fig. 135, metaphase-I chromosomes, drawn separately; Figs. 136—143, anaphase-I with delayed separation, »lagging» and bridges; Fig. 144, anaphase-I with 21 : 21 chromosomes. — Magnification × 2100.

a. *Metaphase-I.* — At metaphase-I the chromosomes in this plant are for the most part very crowded and form a mass, which is shown in Fig. 131. Univalents may often be found outside the plate (Figs. 132—133) and multivalents are observed in all the cells. The multivalents found in Fig. 131 could not be closely analysed, but in Fig. 132 at least one hexavalent occurs, and in Fig. 133 are found a quadrivalent ring and a heptavalent chain. In Fig. 134 a trivalent chain and a hexavalent are found, and in some plates which could not be closely analysed an association of eight chromosomes was observed. *The associations of more than six chromosomes indicate the occurrence of heterozygosity for a segmental interchange.*

Only one metaphase-I plate was fully analysed. It is drawn separately in Fig. 135, which shows the configuration $1_{VI} + 1_{V} + 1_{IV} + 1_{III} + 11_{II} + 2_{I}$. The sex chromosomes are found as bivalents in this plate.

b. *Anaphase-I and later stages.* — At anaphase-I the separation is somewhat disturbed, but the frequency of the disturbances was not closely estimated in this plant. In the great majority of the cells, however, the anaphase was quite regular. At early anaphase the delayed separation of some bivalents (Figs. 136—137) or multivalents is observed and lagging univalents are fairly frequent (Figs. 136—139). Bridges with fragments have been observed in a number of cells (Fig. 140), and two or more bridges with and without fragments have been observed in many cells (Fig. 141). Thus, *this individual is also to be regarded as an inversion heterozygote.* Bridges of some undefined kind (Figs. 142—143) are relatively abundant. Possibly some of them may really be inversion bridges, although the fragment has not been observed, but they may also be caused by some other agents or be the results of the delayed separation of bivalents or multivalents.

The distribution of chromosomes at anaphase-I seems to be fairly regular, as only plates with 21 chromosomes were observed in the 24 cells examined (Fig. 144). Some low frequencies of unequal distribution must, however, occur as the irregularities met with in the anaphases indicate some asymmetrical arrangements.

E. R 0278—41, FROM FJÄLLSTA.

An individual of a sample from Fjällsta, Sundsjö parish in the province of Jämtland. It was of the typical lowland form with broad leaves and about 50 cm. high. The chromosome number was $2n = 42$.

Configuration	Number of cells
$3_{IV} + 15_{II}$	3
$2_{IV} + 17_{II}$	3
$2_{IV} + 16_{II} + 2_{I}$	1
$1_{IV} + 1_{III} + 17_{II} + 1_{I}$	1
$1_{IV} + 19_{II}$	2
$1_{IV} + 18_{II} + 2_{I}$	1
21_{II}	1
$20_{II} + 2_{I}$	1
	Total: 13 cells

a. *Metaphase-I.* — The metaphase-I configurations were analysed in 13 cells with the results given on p. 57, at the bottom.

In no cell was a hexavalent observed, and the highest number of multivalents per cell found in this plant, 3_{IV}, was observed in three cells. The trivalent met with in one cell was not composed of the sex chromosomes.

b. *Anaphase-I.* — The separation at anaphase-I was studied in 100 cells. In 95 cells the separation was quite regular, 1 cell showed three lagging multivalents, and in 4 cells a bridge and a small fragment were observed. Thus, *this plant is heterozygous for a small inverted segment.*

The distribution of chromosomes was studied in 11 cells at anaphase-I. Seven cells showed the distribution 21 : 21, in three cells the distribution 20 : 22 was observed, but in one cell the plates had 19 : 23 chromosomes.

F. R 0628—42, A HYBRID.

This plant originates from a cross between a female plant of the common type found in Denmark and the southern parts of Sweden, introduced in Reykjavík, and a male plant of the ssp. *islandicus* from Fossvogur, S.W. Iceland. Its mode of growth was intermediate between those of its parents. The chromosome number was $2n = 42$.

The metaphase-I configurations were studied in 11 cells with the following results:

Configuration	Number of cells
$2_{VI} + 1_{IV} + 13_{II}$	1
$1_{VI} + 2_{IV} + 14_{II}$	3
$1_{VI} + 1_{IV} + 1_{III} + 14_{II} + 1_{I}$	1
$1_{VI} + 1_{IV} + 16_{II}$	2
$1_{VI} + 18_{II}$	1
$3_{IV} + 15_{II}$	1
$2_{IV} + 17_{II}$	1
$1_{IV} + 19_{II}$	1
	Total: 11 cells

In none of them are higher associations found, and although the plant is a hybrid, its meiotic configurations may be regarded as typical of an autohexaploid.

At anaphase-I 27 cells were examined. Three cells showed a bridge with a fragment. Thus, *the plant is heterozygous for an inversion.*

G. R 0634—42, A HYBRID.

A brother plant to *R 0628—42*. Its chromosome number was $2n = 42$.

The configurations at metaphase-I were analysed in 11 cells with the following results:

Configuration	Number of cells
$1_{IV} + 19_{II}$	6
21_{II}	4
$20_{II} + 2_{I}$	1
	Total: 11 cells

The configurations met with in this plant are very different from those of its brother plant, as no cell with hexavalents is met with. Only bivalents are found in 4 cells, and two univalents are met with in one cell. These differences between the two brother individuals are most possibly due to the heterozygosity of the parents.

At anaphase-I 101 cells were studied in side view, 74 of which proved to be quite regular. In 10 cells one bridge with a fragment was found, 8 cells were found to have two bridges with fragments, and in 6 cells three bridges with small fragments were observed. Thus, *the plant is to be regarded as heterozygous for two inverted segments in different chromosomes* (cf. DARLINGTON, 1937). In 3 cells one lagging bivalent was observed.

H. R 0638—42, A HYBRID.

This plant belonged to the same cross as the two last-mentioned individuals. Its chromosome number was $2n = 42$.

The configurations at metaphase-I were examined in side view in 15 cells, with the following results:

Configuration	Number of cells
$1_{VI} + 3_{IV} + 12_{II}$	2
$1_{VI} + 2_{IV} + 14_{II}$	2
$1_{VI} + 1_{IV} + 1_{III} + 14_{II} + 1_{I}$	1
$1_{VI} + 1_{IV} + 16_{II}$	3
$3_{IV} + 15_{II}$	2
$2_{IV} + 17_{II}$	1
$2_{IV} + 16_{II} + 2_{I}$	1
$1_{IV} + 19_{II}$	1
$1_{IV} + 18_{II} + 2_{I}$	1
21_{II}	1
	Total: 15 cells

One hexavalent was observed in 8 cells, but no cell had more than one hexavalent and four quadrivalents. This must be due to the heterozygosity of the parents.

In 8 anaphase-I cells a bridge with a fragment was observed, but 87 cells were quite regular. In 5 cells a delayed separation of one bivalent was noted. *The plant is to be regarded as an inversion heterozygote.*

I. R 0653—42, A HYBRID.

A hybrid between a female plant from Frösön and a male plant of the ssp. *islandicus* from Fossvogur. Its habit was almost the same as that of its father, but it flowered about one month earlier, like the mother plant. The chromosome number was $2n = 41$.

a. *Metaphase-I.* — As it was exceedingly difficult to find all the configurations in the cells, only eight cells were completely analysed, with the following results:

Configuration	Number of cells
$1_{XII} + 2_{IV} + 10_{II} + 1_{I}$	1
$1_{X} + 1_{VI} + 2_{IV} + 8_{II} + 1_{I}$	1
$1_{VIII} + 1_{VI} + 3_{IV} + 1_{III} + 6_{II}$	1
$1_{VIII} + 1_{VI} + 3_{IV} + 7_{II} + 1_{I}$	1
$1_{VIII} + 1_{VI} + 2_{IV} + 9_{II} + 1_{I}$	2
$2_{VI} + 1_{V} + 2_{IV} + 8_{II}$	1
$2_{VI} + 2_{IV} + 1_{III} + 9_{II}$	1
	Total: 8 cells

As may be seen in the table, associations of more than six chromosomes are met with in 6 of the 8 cells analysed. In one cell an association of 12 chromosomes is met with, and in another cell an association of 10 chromosomes is observed. *The individual is, thus, heterozygous for a segmental inter-*

Figs. 145—147. Meiosis in *R. Acetosella* s.str., *R 0653—42*. — Fig. 145, associations of six, eight, ten and twelve chromosomes from different cells; Fig. 146, a normal cell at prophase; Fig. 147, a dwarf cell at prophase. — Magnification × 2100.

change, and most possibly the interchange is found between chromosomes belonging to groups normally forming two hexavalents, as is evidenced by the occurrence of the two associations mentioned above. Some associations from different cells are separately drawn in Fig. 145.

The odd chromosome is found as a univalent in five cells, but in three cells it is paired with other chromosomes forming a trivalent or a pentavalent. It was sometimes observed as a univalent outside the plate.

Associations of eight, ten and even twelve chromosomes were observed in some other cells, none of which, however, could be completely analysed.

At metaphase-I some dwarf cells without or with one, two or three bivalents were observed, and at prophase such cells were found in a low frequency (Figs. 146—147). These dwarf cells are possibly the result of a »cytomixis» in the pre-meiotic divisions, although no cells with supernumerary chromosomes were observed.

b. *Anaphase-I*. — The separation of the chromosomes was studied in 100 cells, 93 of which showed a regular separation, but in 4 cells a bridge with a fragment was found. *The plant is, therefore, heterozygous for an inversion.* A lagging univalent was found in 2 cells, and in 1 cell a lagging bivalent was noted.

The distribution of chromosomes at anaphase-I was studied in 30 cells.

In 15 cells the distribution was 20 : 21, 9 cells showed 19 : 22, and in 6 cells 18 : 23 chromosomes were counted.

J. R 0656—42, A HYBRID.

A brother plant to the last-mentioned one. Its chromosome number was $2n = 42$.

The configurations at metaphase-I were analysed in side views of 11 cells, with the following results:

Configuration	Number of cells
$1_X + 1_{VI} + 3_{IV} + 7_{II}$	1
$1_{VIII} + 1_{VI} + 3_{IV} + 7_{II} + 2_I$	1
$2_{VI} + 3_{IV} + 9_{II}$	1
$2_{VI} + 2_{IV} + 10_{II} + 2_I$	1
$1_{VI} + 2_{IV} + 14_{II}$	1
$1_{VI} + 2_{IV} + 13_{II} + 2_I$	1
$1_{VI} + 1_{IV} + 16_{II}$	2
$1_{VI} + 18_{II}$	1
$2_{IV} + 16_{II} + 2_I$	1
$1_{IV} + 18_{II} + 2_I$	1
	Total: 11 cells

This plant is likewise heterozygous for a segmental interchange, although the frequency of the different associations differs somewhat from that in the plant *R 0653—42*.

At anaphase-I 85 cells were examined. No irregularities were observed in any cell, and a great number of anaphase-II showed quite regular separation.

K. R 0658—42, A HYBRID.

A plant belonging to the same progeny as the two last-mentioned. Its chromosome number was $2n = 42$.

The configurations found at metaphase-I in 10 cells analysed were as follows:

Configuration	Number of cells
$1_{VIII} + 1_{VI} + 2_{IV} + 10_{II}$	1
$1_{VIII} + 2_{IV} + 13_{II}$	1
$2_{VI} + 2_{IV} + 11_{II}$	1
$1_{VI} + 2_{IV} + 14_{II}$	3
$3_{IV} + 15_{II}$	1
$2_{IV} + 17_{II}$	1
$1_{IV} + 19_{II}$	1
21_{II}	1
	Total: 10 cells

Heterozygosity for a segmental interchange is also met with in this plant, but the frequency of associations and multivalents seems to be somewhat different from that of the brother individuals.

At anaphase-I 100 cells were studied, 92 of which showed regular separation. In 2 cells one bridge and a fragment were observed, and in 2 other cells two bridges with fragments were found. *This indicates heterozygosity for an inversion.* In 4 cells lagging multivalents were observed.

L. R 0794—42, A HYBRID.

A hybrid between the female plant *R 0136—39* and the male *R 0135—39*, both originating from Frösön. Its chromosome number was $2n = 42$.

The configurations met with in 9 cells at metaphase-I were as follows:

Configuration	Number of cells
$2_{VI} + 2_{IV} + 11_{II}$	1
$2_{VI} + 1_{IV} + 13_{II}$	2
$1_{VI} + 2_{IV} + 14_{II}$	1
$3_{IV} + 15_{II}$	1
$2_{IV} + 17_{II}$	1
$1_{IV} + 19_{II}$	2
21_{II}	1
	Total: 9 cells

No association of more than six chromosomes is met with; moreover, the plant seems to be free from univalents at metaphase-I.

100 cells at anaphase-I were examined. 98 cells were quite regular, but in 2 cells a bridge with a very small fragment was observed, *indicating heterozygosity for an inversion.*

M. R 0796—42, A HYBRID.

A plant of the same progeny as the last-mentioned one. The chromosome number was $2n = 42$.

The metaphase-I configurations were analysed in 10 cells with the following results:

Configuration	Number of cells
$1_{VI} + 3_{IV} + 12_{II}$	2
$1_{VI} + 2_{IV} + 14_{II}$	2
$1_{V} + 2_{IV} + 14_{II} + 1_{I}$	1
$3_{IV} + 15_{II}$	1
$2_{IV} + 17_{II}$	2
$1_{IV} + 19_{II}$	2
	Total: 10 cells

The plant shows practically no differences from its brother plant mentioned above.

At anaphase-I 100 cells were examined, 98 of which were quite regular, but 2 cells showed one bridge with a very small fragment. It is assumed that *these bridges are due to the same inversion as the bridges found in R 0794—42.*

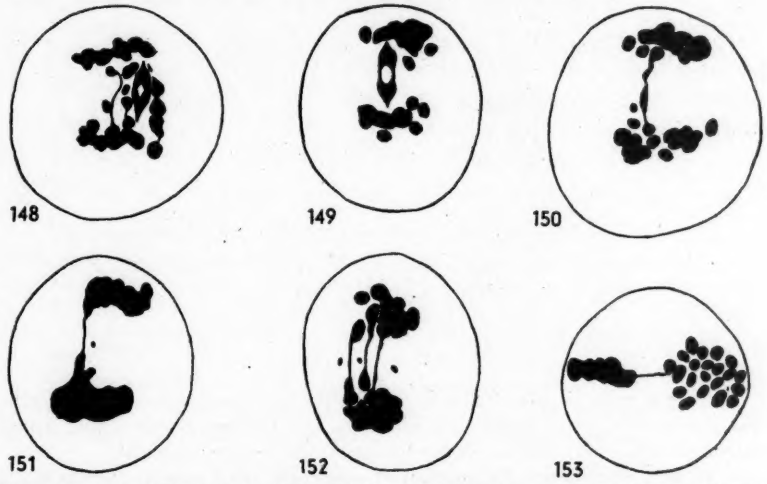
N. R 0839—41, FROM RÄTTVIK.

A tall plant of the main *R. Acetosella* s.str. type from Rättvik in the province of Dalarna. The chromosome number was $2n = 42$.

The configurations of 20 metaphase-I plates are given in the table below:

Configuration	Number of cells
$1_{IV} + 19_{II}$	1
$1_{IV} + 18_{II} + 2_{I}$	1
21_{II}	15
$20_{II} + 2_{I}$	3
	Total: 20 cells

It is assumed that the high frequency of cells with only bivalents met with in this plant is caused by a high degree of heterozygosity.



Figs. 148—153. Meiosis in *R. Acetosella* s.str., *R 0839—41*. — Figs. 148—149, delayed separation at anaphase-I; Figs. 150—151, cells with a bridge and a fragment; Fig. 152, a cell with three bridges with fragments; Fig. 153, metaphase-II with a persistent bridge. — Magnification × 2100.

At early anaphase-I a certain delayed separation was observed in some cells (Fig. 148), and lagging ring-bivalents were rather frequent (Fig. 149). In Fig. 150 a bridge or a multivalent is found. At late anaphase-I 175 cells were examined, 150 of which showed quite regular separation, 11 cells had one bridge and a fragment (Fig. 151), 5 cells had two bridges and two fragments, and in 3 cells three bridges with fragments were observed (Fig. 152). Lagging bivalents were found in 6 cells at late anaphase-I.

The distribution of chromosomes at anaphase-I was studied in 22 cells.

18 cells showed the regular distribution 21 : 21, but in 4 cells the chromosomes were 20 : 22.

At the second division 50 cells were examined. Bridges were found in 6 of these cells, 2 of which had persisted from the first division (Fig. 153).

This individual is assumed to be heterozygous in many pairs of chromosomes, as it shows an unexpectedly high frequency of cells with only bivalents. It shows no detectable heterozygosity for segmental interchanges, but at anaphase-I and -II there is evidence of the occurrence of *heterozygosity for two inverted segments.*

O. R 0854—42, FROM REYKJAVÍK.

This individual belongs to the ssp. *islandicus.* It is a low, repent plant with relatively small leaves, and its flowering period begins about one month later than that of the plants from Scandinavia. The chromosome number is $2n = 42$.

The configurations at metaphase-I were examined in 12 cells with the following results:

Configuration	Number of cells
$1_{VI} + 3_{IV} + 12_{II}$	1
$1_{VI} + 2_{IV} + 14_{II}$	2
$1_{VI} + 1_{IV} + 16_{II}$	2
$3_{IV} + 15_{II}$	1
$2_{IV} + 17_{II}$	1
$2_{IV} + 16_{II} + 2_{I}$	1
$1_{IV} + 1_{III} + 17_{II} + 1_{I}$	1
$1_{IV} + 19_{II}$	3
	Total: 12 cells

No cell with an association of more than six chromosomes is met with, and one hexavalent is found in 5 cells. In one cell one trivalent and a univalent are observed. These do not belong to the sex chromosomes.

In only 50 cells was the anaphase-I examined. In 3 cells one bridge and a fragment were found, and two bridges with fragments were observed in 2 cells, indicating *heterozygosity for one inverted segment.* In 1 cell one lagging quadrivalent was met with, but 44 cells were quite regular.

The distribution of chromosomes at anaphase-I was found to be 21 : 21 in all the 15 cells examined.

P. SUMMING UP.

It is evident from the results of the studies on the meiosis of *R. Acetosella* s.str. that meiotic irregularities of different kinds are more frequent in this autohexaploid than in its tetraploid relative *R. tenuifolius.* The most frequent disturbances observed in the material are

inversions, which are met with in 14 out of the 15 individuals examined. In 2 plants heterozygosity for two inverted segments was observed. Heterozygosity for one segmental interchange was found in 5 individuals. Univalents were met with in a number of plants, and an unexpectedly low number of multivalents, assumed to be caused by a high degree of heterozygosity, was observed in some individuals. All these meiotic irregularities are, however, in full accordance with that expected for a polyploid with different karyotypes differing in segmental interchanges and inversions.

4. MEIOSIS IN RUMEX ACETOSELLA S. STR. × TENUIFOLIUS HYBRIDS.

As will be shown later (pp. 83—84), some hybrid plants were obtained after the cross *R. Acetosella* s.str. ♀ × *R. tenuifolius* ♂. These plants were both males and females, and their somatic chromosome number was $2n = 35$, 21 of which originate from the mother plant (18 autosomes and 3 *X*-chromosomes) and 14 from the father (12 autosomes and 2 *X*-chromosomes in the females, and 12 autosomes and an *XY*-pair in the males.) The meiotic behaviour of two male plants was closely examined, with the following results:

A. R 0661.

The prophase stages of both the hybrids were not closely examined, but the pairing seemed to be almost the same as in the prophase stages of the two parental species, and the »bouquet» stage seemed to be of somewhat longer duration than in the species themselves. At diakinesis most of the chromosomes were paired but no close examination of the pairing was made before metaphase-I.

a. *Metaphase-I.* — At metaphase-I the configurations were completely analysed in 12 cells with the following results. The associations of the sex chromosomes are stated beside each one of the configuration rows.

	Configuration		Number of cells
a	$2_V + 2_{IV} + 2_{III} + 5_{II} + 1_I$	$(XXXY + X)$	1
b	$2_V + 1_{IV} + 2_{III} + 7_{II} + 1_I$	$(XXY + XX)$	1
c	$2_V + 4_{III} + 9_{II}$	$(XXY + XX)$	2
d	$1_V + 2_{IV} + 10_{II} + 2_I$	$(XXXXY)$	1
e	$1_V + 1_{IV} + 2_{III} + 9_{II} + 2_I$	$(XY + XX + X)$	1
f	$1_V + 1_{IV} + 1_{III} + 11_{II} + 3_I$	$(XXY + XX)$	1
g	$1_V + 13_{II} + 4_I$	$(XXXXY)$	1
h	$2_{IV} + 1_{III} + 10_{II} + 4_I$	$(XY + XX + X)$	1
i	$2_{IV} + 1_{III} + 9_{II} + 6_I$	$(XY + XX + X)$	1
j	$1_{IV} + 3_{III} + 9_{II} + 4_I$	$(XXY + XX)$	1
k	$1_{IV} + 1_{III} + 12_{II} + 4_I$	$(XY + XX + X)$	1
			Total: 12 cells

In two cells the pentavalent is composed of the sex chromosomes, but in all the other cells it is composed of autosomes. Univalents are found in all but two cells, but as the frequency of univalents is lower than was expected, a more detailed discussion of each one of the configurations is called for.

As mentioned above, three haploid sets of autosomes and three *X*-chromosomes in the hybrid plants originate from *R. Acetosella* s.str., and two haploid sets of autosomes and one *X* and one *Y* in the male hybrid plants originate from *R. tenuifolius*. A discussion of the pairing properties of the sex chromosomes will be given on a later page, but here the configurations of the autosomes will be considered. The number of autosomes of the hybrid individuals is $2n = 30$ ($18 = 3 \times 6$ from *R. Acetosella* s.str. and $12 = 2 \times 6$

Figs. 154—159. Meiosis in *R. Acetosella* s.str. × *tenuifolius* hybrids. — Figs. 154—155, metaphase-I chromosomes of two cells from *R 0661*, drawn separately; Fig. 156, metaphase-I with a univalent outside the plate; Fig. 157, anaphase-I with delayed separation, bridges and »laggings»; Fig. 158, a cell with a »lagging» univalent; Fig. 159, metaphase-I chromosomes from *R 0664*, drawn separately. — Magnification × 2100.

from *R. tenuifolius*). For the sake of simplicity the autosomes from the hexaploid species will be denoted by the letter *A* and the autosomes from the tetraploid species by the letter *T*.

The configuration *a* (cf. the table) with the autosomes indicated as $2_V + 1_{IV} + 2_{III} + 5_{II}$ is supposed to be formed by both auto- and allosyndesis. Each of the pentaploids is made up of three *A* chromosomes and two *T* chromosomes. The quadrivalent may be formed by two *A* and two *T* chromosomes, or by three *A* and one *T* chromosome, always giving one univalent chromosome of the same set. The trivalents may possibly be formed by autosyndesis from three *A* chromosomes, and then the bivalents are mostly formed by the autosyndesis of three pairs of *T* chromosomes and one pair of *A* chromosomes. It is not unlikely, however, that the bivalents and trivalents will be formed by allosyndesis as well as by autosyndesis, like the penta- and quadrivalents. In both cases two different univalent *A* chromosomes are to be expected. In

cell *a*, however, no univalents are met with (cf. Fig. 154). This phenomenon will be discussed in greater detail below.

The configuration *b*, with $2_V + 1_{IV} + 1_{III} + 6_{II} + 1_I$, is also formed by auto- and allosyndesis in at least the pentavalents and the quadrivalent. Three univalents might be expected, though only one is met with in the cell.

The configuration *c*, with $2_V + 2_{III} + 7_{II}$, would theoretically give two univalents. No univalent is, however, observed.

In the configuration *d*, with $2_{IV} + 10_{II} + 2_I$, six univalents are expected. Only two are met with in the cell.

In cell *e*, with $1_V + 1_{IV} + 2_{III} + 7_{II} + 1_I$ (Fig. 155), three univalents should be met with. Only one is detected.

The configuration *f*, with $1_V + 1_{IV} + 10_{II} + 3_I$, is expected to give five univalent autosomes, only three of which could be discovered.

In cell *g*, with $13_{II} + 4_I$, only the configuration $12_{II} + 6_I$ was expected. Two of the univalents from different pairs must therefore have formed a bivalent.

Cell *h*, with the configuration $2_{IV} + 1_{III} + 8_{II} + 3_I$, also has two univalents less than expected.

Cells *i* and *j*, with $2_{IV} + 1_{III} + 7_{II} + 5_I$ and $1_{IV} + 2_{III} + 8_{II} + 4_I$ respectively, show the expected number of univalents provided only auto- and allosyndesis are assumed to occur.

In cell *k*, with the configuration $1_{IV} + 1_{III} + 10_{II} + 3_I$, the number of univalents expected is five, but only three are observed.

As shown above, both allo- and autosyndesis occur in the hybrid. In ten cells, however, the number of univalents is lower than expected if pairing only occurs between homologous chromosomes from the five sets. In most of the cases two expected univalents seem to form a bivalent, but in cell *d* the four expected univalents seem to form two bivalents. This phenomenon may be accounted for as follows:

Firstly, the basic number of the species may be lower than 6 or 7, for instance, 4 or 5, the number 6 or 7 being produced by a duplication of one or two chromosomes. After undergoing this duplication the chromosomes have differentiated somewhat, and they will only form a chiasma if the completely homologous chromosome is not met with in the cell. This interpretation is rather strained, and if it were correct in this case one of its consequences would be that associations of more than two chromosomes in the diploid species and of more than four in the tetraploid and more than six in the hexaploid would not necessarily be the results of segmental interchange. As the diploid species affords no clear reasons for this interpretation, the present writer supposes that it is not applicable to this material.

The *second* possibility is pairing between small homologous segments in the chromosomes of the different pairs. This possibility,

however, is almost as extraordinary as the previous one, as the pairing of the univalents is very frequent.

The *third* possibility is pairing between fully non-homologous chromosomes, as found, for instance, in some monohaploids (cf. LEVAN, 1942). I am not able to decide whether this possibility is any more likely than the former ones. Maybe the pairing of the expected univalents in this hybrid plant is due to both non-homologous pairing and pairing between small homologous segments in the different chromosomes.

The *fourth* possibility is »pairing» between heterochromatic regions of non-homologous chromosomes. Such »pairing» was postulated by BLEIER (1933) and MCCLINTOCK (1933) and observed by DARLINGTON and LA COUR (1942). The writer is not able to judge the significance of this possibility for our problem, but a certain explanation of it may perhaps be obtained after cold-treatment of the hybrid plants.

In some few cells one or more univalents were found to go to the pole somewhat earlier than the rest. One such cell is depicted in Fig. 156.

b. *Anaphase-I.* — At anaphase-I bridges with fragments, laggings, delayed separation, etc. were observed. One cell with two bridges with fragments, a delayed separation of one multivalent and two lagging univalents is shown in Fig. 157, and one lagging univalent is found in Fig. 158. A close analysis was made of 162 cells, with the following results: 100 cells showed separation without detectable irregularities, but in 62 cells some disturbances were found. One bridge with a fragment was found in 23 cells, in 3 cells two bridges with fragments and in 2 cells three bridges with fragments were observed, indicating heterozygosity for two inverted segments. One lagging chromosome was observed in 32 cells, and two or more laggings were found in 3 cells. In one cell two laggings, two bridges with fragments and the delayed separation of a multivalent were found at anaphase-I (Fig. 157). The lagging univalent divided in 12 cells at anaphase-I.

The distribution of chromosomes was examined in 12 cells at anaphase-I. In 4 cells it was 14 : 21, in 3 cells 17 : 18, 2 cells showed 15 : 20, and 2 cells had 16 : 19 chromosomes. In one cell, however, the distribution 15 : 21 was observed, possibly owing to a divided univalent at anaphase-I.

B. R 0664.

The fixation of this plant was not very successful except at metaphase-I and, in the case of a few cells, at anaphase-I.

The metaphase-I configurations were analysed in 11 cells with the results given on p. 69.

This plant shows almost the same conditions as those found in its brother plant described above. In all but two cells (*d* and *h*) two of the expected univalents have formed a bivalent, and in one cell (*c*) four expected univalents

have formed two bivalents. Allo- and autosyndesis is found in at least some of the cells in this individual also.

	Configuration		Number of cells
a	$2_V + 2_{IV} + 1_{III} + 6_{II} + 2_I$	$(XXY + XX)$	1
b	$2_V + 1_{IV} + 1_{III} + 8_{II} + 2_I$	$(XXY + XX)$	1
c	$1_V + 2_{IV} + 2_{III} + 8_{II}$	$(XXY + XX)$	1
d	$1_V + 1_{IV} + 3_{III} + 7_{II} + 3_I$	$(XXY + XX)$	1
e	$1_V + 2_{III} + 11_{II} + 2_I$	$(XXXXY)$	1
f	$2_{IV} + 2_{III} + 9_{II} + 3_I$	$(XY + XX + X)$	1
g	$1_{IV} + 2_{III} + 11_{II} + 3_I$	$(XXY + XX)$	1
h	$1_{IV} + 1_{III} + 11_{II} + 6_I$	$(XXY + XX)$	1
i	$2_{III} + 13_{II} + 3_I$	$(XY + XX + X)$	1
j	$1_{III} + 14_{II} + 4_I$	$(XXY + XX)$	1
k	$15_{II} + 5_I$	$(XY + XX + X)$	1
			Total: 11 cells

In one cell at metaphase-I two pairs of chromosomes were found to form an accessory plate between the metaphase-plate and one of the poles. In Fig. 159 the metaphase-I chromosomes of one cell are drawn separately.

At anaphase-I only 20 cells were studied, 14 of which were apparently regular; in 5 cells lagging univalents were found and in 1 cell there was a bridge with a fragment.

VI. THE STERILITY OF RUMEX SG. ACETOSELLA.

1. INTRODUCTION.

It is a well-known fact that hybrids between different species or forms of plants and animals are frequently partially or completely sterile. Although the phenomenon of hybrid sterility is now fairly well known, it is not always possible to analyse its primary causes in each case. According to some previous workers (cf. MÜNTZING, 1930), giving detailed accounts on the sterility phenomena in animals and plants respectively, two kinds of sterility, *gametic* and *zygotic*, can be distinguished. Gametic sterility consists in production of non-functional or degenerate gones, and zygotic sterility produces gones which form inviable zygotes. As these terms do not make a clear distinction between the two main categories of sterility causes, the terms *haplontic* and *diplontic* sterility are to be preferred (MÜNTZING, 1930). According to the definition given by MÜNTZING, haplontic sterility is an »absolute or partial inability to produce progeny in a sexual way caused by an absolute or partial lethality of the haplophase», and diplontic sterility is defined as an »absolute or partial inability to produce progeny in a

sexual way caused by an absolute or partial lethality of the diplophase». A detailed discussion on the phenomena in connection with the two new terms is given by MÜNTZING (1930).

According to DOBZHANSKY (1937), the above-mentioned terms should be exchanged for his terms *genic* and *chromosomal* sterility. The former is »due not to dissimilarities in the gross structure of the chromosomes, but to the genetic constitution of the organism», and the latter is due to »structural dissimilarities». Although these two terms in somewhat greater detail account for the nature of the sterility, the present writer prefers the terms haplontic and diplontic as being wider and as including also the borderline cases of genic and chromosomal sterility. Haplontic and diplontic sterility each includes both genic and chromosomal sterility (cf. MÜNTZING, 1930; DOBZHANSKY, 1937).

It has been shown by many investigators that partial sterility is fairly frequent in plants and animals. According to MÜNTZING (1939 a), a direct correlation is often found between meiotic disturbances and the pollen fertility of plants in which the pollen may be divided into two classes: filled grains, which are regarded as fertile and functionable, and empty dead grains. When such a correlation is met with, a haplontic chromosomal sterility is said to be found in the individual. Such a sterility of the pollen grains may also, of course, be caused by genes: haplontic genic sterility. Some plants, however, may show partial or complete sterility of the pollen grains owing to physiological disturbances caused by the diploid tissues of the mother individuals. Then the plant shows a diplontic genic sterility of the gones. Another form of diplontic sterility may be caused by differences in the supply of nourishment of the gones: diplontic physiological sterility. Both the last-mentioned kinds of sterility seem to exist in, for instance, *Prunus persica* (WANSCHER, 1941). The sterility mostly met with in the plant kingdom is of the haplontic chromosomal or genic category.

In the animal kingdom, however, the gametes are mostly found to be morphologically normal and functionable, even if the meiotic behaviour of the individual shows disturbances of different kinds. The individual may, however, be classed as a partially sterile one, as it will give rise to two classes of offspring: the first includes normal zygotes with the regular »make-up» of chromosomes, the second group fails to develop — or shows decrease in vitality — because the gametes which fertilized the eggs were chromosomally defect. The chromosomal sterility met with in the animal kingdom is thus mainly of the

diplontic type. When the genetic constitution of the gametes determines inviability of some part of the zygotes, the sterility is diplontic and genic. Diplontic sterility of various other kinds is met with in the animal and plant kingdoms (cf. MÜNTZING, 1930, 1939 a, b).

Regarding the correlation between meiotic irregularities and sterility in plants, the problem of the distribution of haplontic and diplontic sterility of the chromosomal type may be of great interest. As mentioned above, haplontic chromosomal sterility is the most frequent form within the plant kingdom. Some of the best examples of this are the plants with ring-of-four and about 50 % numerical non-disjunction and about 50 % abortive pollen grains, as found in, e. g., *Zea mays* (MCCLINTOCK, 1931; COOPER and BRINK, 1931; RHOADES, 1931, 1933, and others), *Rosa* (ERLANSON, 1931), *Tradescantia* (SAX and ANDERSON, 1933), *Pisum* (HÅKANSSON, 1931; SANSOME, 1932), *Dactylis* (MÜNTZING, 1937), *Secale* (MÜNTZING, 1939 a; MÜNTZING and PRAKKEN, 1941). According to MÜNTZING and PRAKKEN (l. c.), a part of the sterility met with in rye may, however, be of the diplontic type caused by differences in the supply of nourishment of the cells or other physiological factors. In triploid plants of rye the pollen is »surprisingly good», according to the observations of MÜNTZING and PRAKKEN (l. c). The same fact is observed in triploid plants of *Melandrium album* (WESTERGAARD, 1940) and *M. rubrum* (D. LÖVE, unpubl.) as well as in triploids of *Achnida tamariscina*, which show up to 80 % of morphologically good pollen (MURRAY, 1940 b). In diploid *Melandrium* no correlation is found between the meiotic irregularities and the percentage abortive pollen grains (D. LÖVE, unpubl.), and in *Notonia grandiflora* from the Himalaya (GANESAN, 1939) plants with four-rings showed about 98 % fertility of the pollen grains, but only produced about 27 % of mature seeds. This indicates that the chromosomal sterility met with in *Notonia*, and most probably also in *Melandrium* and *Achnida*, is of the diplontic type as frequently met with in animals. According to the results mentioned below, the species of *R.* sg. *Acetosella* also seem to belong to this category.

2. FERTILITY OF RUMEX ANGIOCARPUS.

The fertility of pollen grains of *R. angiocarpus* was closely studied in 25 male plants. The lowest fertility calculated was 83,2 % in plant *R 0585* with fully normal meiosis, most of the plants, however, showing between 95 and 100 % apparently good pollen grains. The mean value was $96{,}0 \pm 4{,}3$ % good pollen.

The type with segmental interchange showed 99,0 % apparently fertile pollen grains, contrary to expectations based on the studies of the distribution of chromosomes at anaphase-I. In this plant a chromosomal non-disjunction giving the distribution 6 : 8 chromosomes was observed in 51 % of the cells studied, and it is assumed that at least $^2/_3$ of the gones are the results of a numerical and genetical non-disjunction. In the ring-of-four-forming individual of *Notonia* GANESAN (l. c.) observed about 98 % perfect pollen grains, in spite of at least 52 % non-disjunction.

As *R. angiocarpus* is dioecious, no seeds were obtained from the plant with segmental interchange, and the ring-formation was not observed before the plant was dead, so that the germination of pollen grains could not be tested. Unfortunately, no hybrids between this plant and a normal female plant were made in order to study the percentage of filled mature seeds. The seeds of the female plant *R 0582* at the one side in the row of the male with segmental interchange showed, however, an unusually high frequency of yellow, unfilled achenes. Normally, the frequency of unfilled achenes is only 0—5 %, but in *R 0582* it was found to be almost 35 %. Presumably this may be due to fertilization by pollen grains resulting from non-disjunction in *R 0583*. The zygotes, then, show a high degree of abortion, as is also found in *Notonia*.

In the plant heterozygous for an inversion the percentage of apparently fertile pollen grains was about 89 %, or 10 % lower than in the ring-forming individual. This might, of course, be partly connected with the occurrence of the inversion, but as the frequency of bridges was estimated at only about 1,5 %, it is evident that the causes of the higher degree of sterility must be due to some other agents. This suggestion is also supported by the fact that in one of the plants with quite regular meiosis only 83 % of filled pollen grains were calculated.

The type with partial asynapsis showed 89 % of fertile pollen grains. As 20 % of the cells examined in this plant showed disturbances in the meiotic divisions, resulting in great variations in the size of the pollen, it is not impossible that the sterility of this individual is somewhat affected by the irregularities.

The plants with fragment, »fusion» of the »tetrads» and partial failure of the second division showed all about 95 % filled pollen grains. As expected, some few giant pollen grains were observed in the two last-mentioned types.

3. FERTILITY OF RUMEX TENUIFOLIUS.

A. THE OBSERVATIONS.

In spite of the presence of meiotic irregularities such as multivalents, bridges, etc., the pollen of *R. tenuifolius* is fairly good. As to the values from 64 male plants, the mean value of percentage of filled grains is $95{,}2 \pm 3{,}4$ %. Most of the individuals showed between 90 and 100 % good pollen, though fifteen plants showed a lower percentage.

It may be of special interest to estimate the relation between the fertility and the meiotic irregularities found in some of the male individuals investigated.

Plant *R 077—5* proved to be heterozygous for both an inversion and a segmental interchange. At anaphase-I irregularities were observed in about 21 % of the cells, and unequal distribution of chromosomes was noted in about 10 % of the cells. The pollen fertility was almost perfectly good as 96 % of filled pollen grains were calculated.

R 084—42 showed a somewhat low frequency of univalents and trivalents, and multivalents were found in 85 % of the cells. At anaphase a very low frequency of bridges and of univalents was observed, and only the regular distribution 14 : 14 was met with. The pollen fertility was about 98 % of filled grains.

R 0108—4 showed lower frequencies of multivalents than were found in *R 084—42*. Its anaphase-I is perfectly regular and the distribution of chromosomes observed was always 14 : 14. The fertility of the pollen was 95 % good.

The individual *R 0109—4* showed trivalents and univalents in 40 % of the cells. A low frequency of lagging univalents was found at anaphase-I. The plant is apparently free from inversions and segmental interchanges. Its pollen fertility was, however, only 47 % good.

R 0110—42 is heterozygous for segmental interchange but free from inversion bridges. The distribution of chromosomes at anaphase-I was somewhat irregular, as 1 cell out of 8 examined showed the distribution 13 : 15. The pollen fertility was about 86 % good, but some variation in the size of the grains was observed.

In plant *R 0111—42* 95 % of the cells studied had one or more multivalents, and univalents were found in 20 % of the cells. The plant showed bridges in 5 % of the anaphases, and the distribution of chromosomes at anaphase-I was quite regular. The pollen was about 82 % fertile.

The individual *R 0144—2* was heterozygous for a segmental interchange, and trivalents and univalents were met with in some cells. The anaphases were quite regular. The pollen proved to be about 90 % fertile.

R 0188—42 showed multivalents in 95 % of the cells, and in 20 % of the cells univalents were found. A bridge with a fragment was observed in 10 % of the anaphases, and the distribution 13 : 15 was noted in 3 cells out of 16 examined. The pollen fertility was found to be 98 % good.

In *R 0604—42* multivalents were found in only 60 % of the cells, and in 25 % of the cells trivalents and univalents were met with. Laggings and

delayed separation were observed at anaphase in a low frequency. The fertility was only 78 % good.

R 0609—42 showed a rather high frequency of »cytomixis» and other physiological disturbances. Multivalents and univalents are met with in a number of cells, and at anaphase inversion-bridges are met with. Variation in chromosome number is detected in some cells at metaphase and anaphase. The pollen fertility of the plant was about 70 % of filled grains.

In the individual *R 0610—42* 90 % of the cells showed multivalents, and univalents and trivalents were observed in 30 % of the cells. At anaphase bridges were observed in two out of 37 cells. The fertility of the pollen grains was about 94 % good.

R 0615—42 had trivalents and univalents in 55 % of the cells, and multivalents were met with in all but 5 % of the cells. In 6 % of the cells anaphase-bridges were observed. The pollen showed 98 % fertile grains.

R 0804—42 was found to be heterozygous for two segmental interchanges and one inversion. At metaphase univalents were found in all but one of the cells examined, and some disturbances were met with in 24 % of the cells at anaphase. The pollen fertility was found to be 99 % good.

R 0814 showed a high degree of meiotic irregularities. It was heterozygous for inversions and segmental interchanges, and a high frequency of laggings was met with in both divisions. The pollen was 78 % good.

R 0829—42 was heterozygous for segmental interchange, but the distribution of chromosomes at anaphase was not studied. No bridges or laggings were observed at anaphase. The pollen was 99 % good.

Plant *R 0831—42* showed heterozygosity for segmental interchange, and multivalents of different kinds were found in 95 % of the cells examined. In 35 % of the cells associations of more than four chromosomes were met with, and univalents were observed in 55 % of the cells. At anaphase a bridge with a fragment was found in 7 % of the cells, and in 14 % of the cells some irregularities were observed. 28 % of the cells showed the distribution 13 : 15 at anaphase. The pollen fertility of this plant is expected not to be higher than 72 % good, if only the irregularities in chromosome number at anaphase are used for the calculation, but the fertility was found to be 79 % good.

R 0834—42 was free from associations of more than four chromosomes, but univalents were found in 45 % of the metaphase-I examined. 3 % of the cells showed laggings at anaphase. The pollen was 95 % good.

Plant *R 0836* was heterozygous for an inversion and showed some irregularities in about 21 % of the anaphases examined. Its fertility was 94 % good.

R 0844—42 showed a rather high frequency of multivalents and univalents, and at anaphase no irregularities were detected. The pollen was 90 % good.

R 0847—42 was heterozygous for two segmental interchanges, and in all the cells studied multivalents or associations of more than four chromosomes were observed. Univalents were fairly frequent, but no irregularities were found in the separation of the chromosomes at anaphase. In 45 % of the cells the distribution of chromosomes at anaphase was not the regular one. The pollen was about 83 % good.

As demonstrated above, only small differences have been found in the fertility of the plants with different degrees of meiotic irregularities. Although some of the plants with a high frequency of meiotic disturbances apparently show a somewhat diminished percentage of fertile pollen grains, some other plants show a high degree of fertility and a fairly high frequency of meiotic disturbances. In plant *R 0847—42*, for instance, which is heterozygous for two segmental interchanges, at least 45 % of abortive pollen grains were expected, but only 17 % of pollen sterility were detected. On the other hand, the highest degree of pollen sterility was met with in plant *R 0109—4* with only a low frequency of meiotic irregularities. Thus, *in R. tenuifolius no definite correlation is found between pollen sterility and meiotic disturbances.*

The fact that no plant with a deviating chromosome number was found in the material of this species, in spite of the evident occurrence of pollen with 13 and 14 chromosomes, was interpreted in a working hypothesis as being due to the elimination of either gones or zygotes with deviating chromosome numbers. If the gones are eliminated, the chromosomal sterility of *R. tenuifolius* must be of the haplontic chromosomal type, but if the zygotes are eliminated, the sterility must be of the diplontic chromosomal type, as found in *R. angiocarpus*. The very fact that no certain correlation was detected between the frequency of filled pollen grains and meiotic irregularities indicated the second alternative, but as it was not impossible that a number of the filled pollen grains would fail to germinate and therefore really be sterile, closer investigations were needed into the germination of pollen grains as well as into the progeny of crosses between female plants and a male with a number of disturbances at meiosis.

B. GERMINATION EXPERIMENTS WITH POLLEN.

In the summer of 1941 the first attempts to germinate pollen of different *Rumex* species were made by the writer. All the experiments to germinate it by the usual method, i. e. in saccharose solutions of different concentrations on 1 to 5 % agar or 10 % gelatine proved a failure. In some cases a low frequency of pollen grains germinated and budded, but in most of the solutions they did not germinate at all. As the stigmas of *Rumex* are relatively very small, no experiments with stigmas or a decoction of stigmas were made. In accordance with the methods described by KUHN (1937), experiments were also made with a very thin substratum, but although the results obtained were better than those first-mentioned, they were not satisfactory.

In the summer of 1942 the experiments continued. The method used on this occasion was the same as that used by KOKHANOVSKAYA (1939), who

studied the germination of the pollen of flax. In the middle of a large cover-glass was placed a small flat drop of substratum of 1, 1,5 or 2 % agar or 10 % gelatine. When the drop had become dry, some pollen was placed on it with nippers and beside the drop were placed a few small drops of a solution of saccharose of a definite concentration ranging from 0,5 to 2,0 *n*. The drop of substratum and the drops of solution did not come into contact with each other. After this procedure the cover-glass was inverted and placed on the frame (the length of the sides 12 mm.; height about 4 mm.) of a moist chamber. The frame was placed on an object-glass and sealed to it and the cover-glass with vaseline. The chambers were placed on the work-table in a laboratory facing E.S.E. and the temperature during the experiments was between 15 and 20° C.

A uniform germination was obtained only on the drops of agar, and in some of the experiments germination began within 10 minutes. The most rapid and most uniform germination was observed in groups at the edge of the dry drop, as was also observed by KOKHANOVSKAYA (l. c.), next to a 1,5—1,8 *n* solution of saccharose. In consequence of this the pollen of the plants examined was germinated next to the last-mentioned solutions.

As the method is somewhat tedious, only a few plants were tested. One plant with almost regular meiosis and about 98 % of good pollen was tested with the result that almost all the filled pollen grains (96 %) were found to germinate. Another plant with only 74 % good pollen but with no serious disturbances at anaphase-I showed a germination of 98 % of the filled grains. Plant *R 0609—42* with cytomixis and only 70 % of filled grains showed a germination of only 90 % of the filled grains.

The germination power of the pollen in the plants with associations of more than four chromosomes and with an unexpectedly high percentage of filled grains is of special interest, as it might be assumed that a number of the filled grains would be unable to germinate. Accordingly, three such plants were studied.

R 0829—42 was heterozygous for a segmental interchange, but apparently free from inversions and other disturbances at anaphase. 99 % of filled pollen grains were calculated. Of the filled grains 94,5 % were found to germinate.

R 0831—42 was heterozygous for segmental interchange and an inversion. 28 % of the cells showed the distribution 13 : 15 at anaphase-I. The pollen fertility was calculated as 79 % of filled grains. About 98 % of the filled grains were found to germinate.

R 0847—42 was heterozygous for two segmental interchanges and in 45 % of the cells irregularities in the distribution of chromosomes at anaphase-I were observed. The expected percentage of filled pollen

grains was about 55 %, but the observations showed about 83 % filled grains. Of these practically all (165 out of 167 examined) were found to germinate.

The results of the germination experiments strongly indicate that pollen grains with deviating numbers of chromosomes are able to germinate. These results further indicate that the pollen sterility met with in some of the individuals is not of the chromosomal type, and they may be regarded as strongly supporting the hypothesis that the chromosomal sterility of *R. tenuifolius* is mainly of the diplontic type.

C. CROSS EXPERIMENTS.

A number of different cross experiments have been made between plants from different localities, and in order to ascertain whether any deviations from the normal somatic chromosome number occurred in the offspring, the chromosome number of all the plants was determined. No plant with more or less than 28 chromosomes has as yet been found in the material.

In some of the crosses, however, a rather high frequency of yellow, unfilled achenes was observed. In one of the crosses the father individual, *R 077—4*, was heterozygous for segmental interchange and showed the distribution 13 : 15 in 10 % of the cells examined. 12 % of the seeds from the cross proved to be unfilled. This fact may be considered to lend good support to the suggestion that a lethality of zygotes with a deviating number of chromosomes is the main cause of the total lack of plants with more or less than 28 chromosomes in *R. tenuifolius*.

4. FERTILITY OF RUMEX ACETOSELLA S. STR.

A. THE OBSERVATIONS.

The fertility of the pollen grains of this species was found to be almost the same as that of the two above-mentioned species. The percentage of filled grains was examined in 68 male plants, only six of which showed a lower value than 90 %. The mean value of apparently good pollen grains was $96{,}8 \pm 2{,}4$ %.

Nor can any relation be discovered between the occurrence of meiotic irregularities and a decrease in the percentage of filled pollen grains in this species, as will be seen upon comparing the meiotic behaviour and the pollen fertility of the male plants investigated.

Plant *R 0133—39* was found to have $2n = 43$ chromosomes. A low frequency of multivalents was met with at metaphase-I, and at anapase-I 11 % of the cells showed inversion bridges, and 15 % of the anaphases showed

some disturbances. In 20 % of the cells the distribution was 20 : 23, but in 80 % it was 21 : 22. The fertility of the pollen was 98 % good.

In the individual *R 0135—39* multivalents are found in all the cells examined, and an inversion bridge is met with in 10 % of the anaphase-I cells. The distribution of chromosomes was found to be irregular (20 : 22 or 19 : 23) in about 36 % of the cells. The pollen showed about 97 % of filled grains.

R 0138—39 was an intersexual plant with heterozygosity for a segmental interchange and an inversion. Multivalents are met with in all the cells, and 3 % of the anaphases proved to be irregular. The pollen fertility was about 98 % good.

The pollen fertility of *R 0140,* the plant with heterozygosity for an inversion and a segmental interchange, has been tested twice. The pollen fertility was 92,8 % good when tested in June, but only 86,2 % when examined in July after a period of extremely high temperature and drought. This difference must be due to physiological disturbances only.

The individual *R 0278—41* showed a low frequency of multivalents and univalents at metaphase-I, and in 5 % of the anaphases studied some disturbances were met with. The plant is an inversion-heterozygote. Irregularities in the distribution of chromosomes at anaphase (20 : 22, 19 : 23) were met with in about 36 % of the cells. The pollen was about 93 % good.

In all the metaphase-I cells of *R 0628—42* multivalents were found, and at anaphase evidences were observed indicating heterozygosity for an inversion. The pollen was about 95 % good.

R 0634—42 showed a low frequency of multivalents and univalents at metaphase and in about 24 % of the anaphase-I cells that were studied, bridges and fragments were found, indicating heterozygosity for two inversions. The pollen showed 99 % of filled grains.

The individual *R 0638—42* showed a rather high frequency of multivalents and a low frequency of univalents. 13 % of the anaphases showed some irregularities. The pollen was about 98 % good.

R 0653—42 was heterozygous for segmental interchange and an inversion. Irregularities in the separation were observed in 7 % of the cells at anaphase-I, and the distribution of chromosomes was irregular (19 : 22, 18 : 23) in 50 % of the cells studied. The plant had $2n = 41$ chromosomes. The pollen was found to be 93 % good.

Plant *R 0656—42* was likewise heterozygous for segmental interchange, but no irregularities were met with in the separation of the chromosomes at anaphase-I. Its pollen fertility was about 99 % of filled grains.

Plant *R 0658—42* was heterozygous for segmental interchange and an inversion, and some disturbances were observed in 8 % of the cells at anaphase. Its pollen fertility was perfectly good, as no unfilled grains were observed.

In the individual *R 0794—42* multivalents were rather frequent at metaphase, and it was heterozygous for a small inversion disturbing 2 % of the anaphases. Its pollen fertility was about 90 % good.

Plant *R 0796—42* showed the same frequency of disturbances as the last-mentioned one. Its fertility, however, was about 98 % good.

R 0839—41 showed multivalents in only 10 % of the cells examined, and

at anaphase-I some irregularities were met with in 14 % of the cells. In about 18 % of the cells the distribution of chromosomes was found to be irregular (20 : 22). The pollen fertility was 95 % good.

Plant *R 0854—42* showed a high frequency of multivalents. It was heterozygous for an inversion and irregularities were observed in 12 % of the cells at anaphase. The distribution of chromosomes at anaphase seems to be completely regular. Its pollen fertility was about 98 % good.

As shown above, no correlation can be found between pollen fertility and meiotic disturbances in this species either. This may be interpreted as being due partly to the high degree of polyploidy, pollen with somewhat deviating numbers being able to germinate and fertilize the ovules, as has also been found in a number of other polyploid species (cf. DARLINGTON, 1937; MÜNTZING, 1937). However, the results obtained here and from the studies of *R. angiocarpus* and *R. tenuifolius* as well as the total lack of types with 40 or 44 ($= 42 \pm 2$) chromosomes in the hexaploid species may be interpreted in the same way, i. e. as an indication of the occurrence of diplontic chromosomal sterility. Owing to their polyploidy, types with 42 ± 1 chromosomes are able to survive, but if the deviation of chromosomes is larger the zygotes will die. This hypothesis is further supported by the experiments mentioned below.

As WANSCHER (1941) has shown, in *Prunus persica* a wide range of variation in the fertility of the pollen grains is found between different flowers on different sprouts of the same tree. Such variation is also found in rye, according to MÜNTZING and PRAKKEN (1941). In order to ascertain whether such a variation was also met with in *R. Acetosella* s.str., two plants, one with about 85 % good pollen in the flowers previously analysed, and another with almost perfect (ca. 100 %) fertility, were closely studied.

Whole stems of both the plants were placed in a glass on the work-table, and the fertility of two or three flowers from each nodium from the top and down to the first flowers was systematically examined. The fertility was found to vary by a few per cent, but no correlation could be detected between fertility and the place of the flower on the stem.

The pollen fertility was, however, found to vary somewhat more widely between two observations at two different times of development. As a rule the pollen fertility of the plants is determined in June, at the time of the main flowering of the species, but when the same plant was studied in July or after a long dry period in June, the

fertility was found to be somewhat lower in every plant. Thus, the fertility may be somewhat affected by the supply of nourishment, temperature, etc., although no differences could be observed between different flowers at the same time.

B. GERMINATION EXPERIMENTS WITH POLLEN.

The germination power of the pollen from four individuals with different fertility was studied after the same method as the pollen of *R. tenuifolius*.

One of the plants, *R 0839—41*, showed 95 % of filled pollen grains, but only about 80 % good pollen were expected from the observations at meiosis. 99 % of the filled grains germinated.

The second plant was *R 0854—42* with about 98 % good pollen and 12 % of disturbed anaphases. All the filled grains germinated.

The third plant was *R 0653—42* with 93 % good pollen in spite of 50 % irregular distribution of chromosomes at anaphase. 98 % of the filled grains germinated.

The last plant was *R 0658—42* with 100 % good pollen and heterozygosity for both an inversion and segmental interchange. 99 % of the pollen were found to germinate.

These results from the germination experiments show clearly that pollen with deviations in the chromosome number is able to germinate also in the hexaploid species *R. Acetosella* s.str.

C. CROSS EXPERIMENTS.

In order to control whether types with $\pm$ more than one extra chromosome would survive, a pollination was made of a female plant with $2n = 42$ chromosomes by the male plant with $2n = 41$ chromosomes and considerable irregularities in the distribution of chromosomes at anaphase-I. According to the observations made on the meiosis of the father plant, it will form about 25 % of pollen with $n = 20$ and $n = 21$ chromosomes respectively, in 15 % of the cells the numbers will be $n = 19$ or $n = 22$, and the numbers $n = 18$ and $n = 23$ will be found in every 10 % of the pollen grains. As was shown by the germination experiments, almost all the pollen grains which proved to be about 93 % good will germinate. If the distribution 21 : 21 were found in almost all the female gones and all the possible combinations were to survive, the anticipated distribution of chromosome numbers in the offspring would be as follows: 25 % of the plants

with 2n = 42 (21 + 21), 25 % with 2n = 41 (21 + 20), 15 % with 2n = 40 (21 + 19), 15 % with 2n = 43 (21 + 22), 10 % with 2n = 39 (21 + 18), and 10 % with 2n = 44 (21 + 23) chromosomes.

The cross resulted in 43 filled and only 8 empty seeds. The filled seeds were sown and gave rise to 41 plants. The chromosome number of these plants was determined in the root-tip cells. 30 individuals had the number 2n = 42, 9 plants had 2n = 41, and in 2 plants the chromosome number was 2n = 43. *No other chromosome numbers were found in this material*; moreover, the proportion of individuals with deviating numbers is different from what might be expected. A high frequency of lethality seems to be found in the zygotes with 2n = 41 and 43 chromosomes, and the total lack of plants with 2n = 39, 40 and 44 chromosomes strongly indicates that the working hypothesis is correct. *The chromosomal sterility of R. Acetosella is most probably of the diplontic type.*

5. FERTILITY OF RUMEX ACETOSELLA S. STR. × TENUIFOLIUS HYBRIDS.

From the interspecific cross between the hexaploid and the tetraploid species there were obtained eleven plants, nine of which were males. As shown in the chapter on the meiosis of these plants, the reduction division is unexpectedly regular, but at anaphase-I cells were found with 14 : 21, 15 : 20, 16 : 19, 17 : 18, etc. chromosomes.

The apparent pollen fertility of the nine hybrid plants was almost as good as that of the parent species, the mean value being $93{,}2 \pm 4{,}8$ % of filled grains. All the plants showed more than 90 % good pollen, except one, which had 89 % of filled grains. In all the plants there was a wide variation in the size of the pollen grains.

Germination experiments were made with three plants showing respectively 89, 92 and 97 % of filled grains. The plant with 89 % good pollen showed germination of 94 % of the filled grains, in the plant with 92 % fertility a germination of 95 % of the filled grains was recorded, and the plant with 97 % of filled grains showed germination of 89 % of the apparently good grains. Thus, *the fertility of the pollen of the hybrids with 2n = 35 chromosomes is in fact almost as good as that observed in the parent species.* No cross experiments with the pentaploid plants have as yet been made, but *the two sister plants of the progeny, growing side by side with the male pentaploids, gave not a single seed after free pollination*, although they bore numerous flowers. These facts are interpreted as being due to diplontic

chromosomal sterility eliminating zygotes with numbers deviating from a multiple of 7. When the chromosome number is high, some smaller deviations may be found, but as the frequency of the euploid numbers 28, 35, and 42 (41, 43), after pollination of a pentaploid plant with pollen from another pentaploid, will be very low, the total lack of seeds from the female hybrids is just what might be expected.

6. CONCLUSIONS.

The pollen sterility met with in some of the plants of *R.* sg. *Acetosella* is very possibly not due to meiotic disturbances. It is presumed to be due to some physiological irregularities in the diploid tissues of the plant, i. e. to be of a diplontic physiological nature. The chromosomal sterility of the group also seems to be of the diplontic type, contrary to almost all previous results from the plant kingdom. This hypothesis is strongly supported by the observations on the meiotic behaviour of different plants of the different species, and their pollen fertility. It is also largely confirmed by the results of the germination experiments of the pollen grains. Last but not least, the total lack of plants with deviating chromosome numbers in the tetraploid species, the lack of progeny from the pentaploid females, as well as the lack of the expected numbers in the progenies of males with known disturbances in the distribution of chromosomes at anaphase, etc. are almost inconceivable without the hypothesis as to the elimination of zygotes with a deviating number of chromosomes.

As mentioned above (p. 71), diplontic chromosomal sterility is met with in *Notonia*, and possibly also in *Melandrium album* and *rubrum* and *Achnida tamariscina*. It must be more than a mere coincidence that three of the four plant genera with diplontic chromosomal sterility are dioecious and belong to the same category of sex determination (cf. below). The dioecious plants are more like the animals than other plants, and, as mentioned above, the chromosomal sterility of the animals is mostly of the diplontic type. Not all dioecious plants, however, show this kind of sterility. In *R.* sg. *Acetosa* sect. *Euacetosae*, for instance, plants with chromosomal disturbances show directly proportional sterility of the pollen grains (YAMAMOTO, 1934, 1938; LÖVE, unpubl.). The dioecious species of *Euacetosae*, however, belong to a different type of sex determination from the three above-mentioned groups. Can it be that the differences in sterility are somewhat correlated with the differences in sex mechanism of these groups?

A closer discussion on the chromosomal sterility in plants and

animals is found, for instance, in the works of MÜNTZING (1930, 1939 a), DOBZHANSKY (1937), GEITLER (1937, 1938), and others.

VII. INTERSPECIFIC CROSS EXPERIMENTS AND THE POSSIBLE OCCURRENCE OF AGAMOSPERMY.

1. HYBRIDIZATION EXPERIMENTS.

The author has made some efforts to obtain hybrids between the three species mainly investigated. The following results were obtained:

R. angiocarpus × *R. tenuifolius.* — Some shoots of three female plants of the diploid species were isolated and pollinated with pollen from a male tetraploid. The cross gave nine seeds, all of which were without any embryo.

R. tenuifolius × *R. angiocarpus.* — Three reciprocal pollinations were made at the same time as the above-mentioned. Three shoots of a female plant were isolated and pollinated with pollen from the diploid, but no seeds were obtained.

R. angiocarpus × *R. Acetosella s.str.* — A pollination was likewise made with three different individuals of the diploid species as mother plants and with pollen from the hexaploid species. The total sum of 30 seeds was obtained. Ten of them were controlled and proved to be without any embryo. The other twenty were sown, but as none of them grew, they, too, were possibly without any embryo.

R. Acetosella s.str. × *R. angiocarpus.* — The reciprocal pollination was made on only one plant with a very large number of flowers. No seeds were obtained.

R. tenuifolius × *R. Acetosella s.str.* — The pollination of a number of *R. tenuifolius* females with pollen from different *R. Acetosella* s.str. males did not succeed in any seed.

R. Acetosella s.str. × *R. tenuifolius.* — This reciprocal cross was made on three different female plants with $2n = 42$ chromosomes. One of the plants yielded 14 seeds, 11 of which germinated and gave rise to 9 males and 2 females. These plants were morphologically more like *R. Acetosella* s.str. than *R. tenuifolius.*

The chromosome number was determined in all the plants, and it was found to be exactly $2n = 35$, i. e. the pentaploid number expected from a successful hybridization between a tetraploid and a hexaploid. The low number of seeds is possibly due to a high frequency of zygotic lethality. The wide difference in the number of males and females, however, is not so easily explainable. When in F_1 offspring of crosses between two different animals (with the same number of chromosomes) »one sex is absent, rare or sterile, that sex is the heterozygous sex»

(HALDANE, 1922), and according to DOBZHANSKY (1937) this rule seems to be correct in most animals studied so far. As only 11 plants from one cross of *R. Acetosella* s.str. × *R. tenuifolius* have been obtained as yet, it is not impossible that the excess of males is a mere coincidene. The phenomenon needs to be more closely examined in a greater number of crosses.

The results of the interspecific cross experiments show that the barriers between the species of *R.* sg. *Acetosella* are mainly bars of incompatibility, as no seeds were obtained from most of the crosses. Such barriers are met with in a number of autopolyploid series (cf. MÜNTZING, 1936, 1937, 1939 b). The seeds without embryo obtained in some of the hybridization experiments may perhaps be due to the fact that zygotes with an unbalanced number of chromosomes die, this being the most probable explanation of the lack of deviating numbers in the progeny from males with grave disturbances of the meiosis. The viability of the pentaploid offspring may be explained on the basis of the high euploid chromosome number giving a high number of homologous chromosomes.

2. POSSIBILITIES OF THE OCCURRENCE OF AGAMOSPERMY.

The results mentioned above must also be regarded in the light of the fact that according to some authors agamospermy may occur in the genus *Rumex*. According to DUDGEON (1918), it might be found in *R. crispus*, and ROTH (1906) and LÖVE (1942 a) have found the same possibility in *R. Acetosa*. Last but not least, MURBECK (cf. NORDSTEDT, 1907, and in a personal communication) has shown that the possibility is not excluded that agamospermy may occur within *R. angiocarpus*, the diploid species in the present investigation.

The present writer (LÖVE, 1942 a) has stated that in about 40 % of the female plants of the dioecious *R. Acetosa* studied, agamospermy occurs in a rather low frequency, and it is not impossible that pseudogamy might be found in this species. Differences are found between female plants from different localities, and some karyotypes seem to be more agamospermous than others (unpubl. data).

MURBECK (l. c.) worked with material of *R. angiocarpus* from Herzegovina. He cultivated some plants, *all females*, in the Botanical Garden at Lund and *obtained a number of seeds*, though no male individuals were found. As it was not entirely out of the question that pollen from *R. tenuifolius* or *R. Acetosella* s.str. had fertilized the ovules, MURBECK isolated a female individual of *R. angiocarpus* the next summer. *The isolated plant yielded some seeds*, of which only a few grew. Unfortunately, MURBECK was not able to continue the experiments, but the possibility of a real agamospermy in his material is, according to the views of the present writer, very large.

On the basis of the above-mentioned results obtained by other in-

vestigators as well as by the author himself in *R. Acetosa*, the author suggested that the possibility of agamospermy in the group *Acetosella* was not excluded. Therefore, a number of female plants of *R. angiocarpus*, *R. tenuifolius*, and *R. Acetosella* s.str. were isolated. *None of these isolations, however, yielded any seeds.* Then the possibilities of pseudogamy were tested by pollinations with pollen of *R. Acetosa*, *R. thyrsiflorus*, and *Rheum undulatum*. *Nor did these experiments yield any seeds in R. angiocarpus and R. tenuifolius, but the pollination R. Acetosella s.str.* × *R. Acetosa gave 6 seeds, four of which germinated.* All the plants have the normal hexaploid chromosome number and they are much like their mother plant. *They are all females.* In view of the last-mentioned fact they may have resulted from pseudogamous development, although the possibility can never be fully excluded that they are the results of an unsuccessful isolation.

To summarize the results mentioned above, it may be concluded that in my material of *R. angiocarpus* and *R. tenuifolius* no indications of the occurrence of agamospermy have as yet been found. In the hexaploid species, however, pseudogamy may possibly exist, and according to the results obtained by MURBECK, it is not entirely out of the question that agamospermy may also occur in some forms of the diploid species.

VIII. SEX DETERMINATION.

1. INTRODUCTION.

The problem of sex determination has been one of the most discussed topics since the first scientific publications in botany and zoology. Philosophical and experimental workers failed, however, to find an adequate explanation of the mechanism of sex determination until the beginning of the 20th century. Comprehensive surveys of the genetical problem of sex determination with reference to the genetical and cytological basis of sex determination are found, e. g., in the works of WETTSTEIN (1936), HARTMANN (1939), HERTWIG (1939), BRIDGES (1939), ALLEN (1940), and KUHN (1942).

Introductory studies of the cellular mechanism of sex determination were made by HENKING (1891) and MCCLUNG (1902), who both studied differences in the morphology of one chromosome pair in the two sexes of insects. The classical investigations by WILSON (1905) into these differences in the bugs *Lygaeus* and *Protenor* threw light on the cytological mechanism which leads to the two kinds of individuals, females and males. The chromosomes which were found to differ in the male and female individuals were named allosomes or sex chromosomes.

In plants the occurrence of sex chromosomes was established in 1917, when ALLEN demonstrated them in the liverwort *Sphaerocarpus*. In dioecious

flowering plants sex chromosomes were observed for the first time in 1922, when BLACKBURN and HARRISON reported that »some evidence exists of the presence of an unequal chromosome pair in the male plants» of *Populus tremula* and some *Salix* species. Definite observations of the sex chromosomes in flowering plants were, however, made in the following year by SANTOS (1923) in *Elodea canadensis*, KIHARA and ONO (1923 a, b) in *Rumex Acetosa*, BLACKBURN (1923) in *Melandrium album*, and by WINGE (1923) in *Melandrium album*, *Humulus japonicus*, *Humulus Lupulus* and *Vallisneria spiralis*. Since then a great number of plants have been found with sex chromosomes. Almost all the cases hitherto published are listed by ALLEN (1940).

The problem of sex chromosomes in *Rumex* sg. *Acetosella* has been one of the most obscure cases reported. According to MEURMAN (1925 a, b), the male plants had $2n = 41$ chromosomes, three of which form a tripartite chain at metaphase-I and anaphase-I. As this chain seemed to be somewhat similar to that found in *R. Acetosa* and *R. thyrsiflorus*, MEURMAN identified them as the sex chromosomes. He supposed that the female plants had the somatic chromosome number $2n = 42$ and four *X*-chromosomes, but that the sex chromosomes of the male individuals were $X_1 + X_2 + Y$. As will be mentioned on a later page, this suggestion if somewhat modified closely resembled the results obtained by the present writer, although the tripartite complex observed by MEURMAN certainly did not always represent the sex chromosomes. KIHARA (1925, 1927 a, b, 1929) found the numbers $2n = 41$, 42 and 43 in the males, but in the female plants studied by him the number was $2n = 42$ chromosomes. As described above, KIHARA observed multivalents in all but one plant at meiosis. He concluded that the tripartite chain described by MEURMAN corresponds to a quadrivalent with one chromosome lacking. Therefore, no evidence has been obtained as to the occurrence of sex chromosomes in *R. Acetosella* s.str., according to the views of KIHARA.

Later works mentioning sex in relation to chromosomes in *R. Acetosella* have been published by ONO (1930), who found $2n = 41$ chromosomes in an intersexual plant, and by JENSEN (1936 a, b), who seems to have observed a tripartite chain at anaphase-I as described by MEURMAN (1925 a, b). He concludes, however, that these multivalents at anaphase have nothing to do with the sex determination of *R. Acetosella* but merely indicate the hybrid origin of this hexaploid species.

The difference in results obtained by previous workers may be somewhat elucidated if it is pointed out that the hexaploid *R. Acetosella* s.str. is a very unsuitable material for studying the sex chromosomes, as its chromosomes are numerous and small and more than one

multivalent can be formed in each pollen mother-cell at metaphase-I. Furthermore, the conclusions drawn by MEURMAN might be somewhat influenced by the mechanism found by him in *R. thyrsiflorus*, and the conclusions drawn by KIHARA may be somewhat affected by his having possibly expected to find only one pair of sex chromosomes or only one multivalent chain with somewhat unequal chromosomes. As will be seen later on, the sex mechanism existing in this hexaploid species is based upon the *XX—XY* mechanism found in the diploid species *R. angiocarpus*.

Before a more detailed account is given of the results obtained by the present writer, some observations will be made on the fundamental experiments which have been made in order to establish the location of the sex-determining genes.

a. *Drosophila and Rumex Acetosa.* — The first attempts to solve this problem were made by BRIDGES (1922, and elsewhere, cf. 1939) in his studies of *Drosophila melanogaster*. He investigated principally triploid, tetraploid and aneuploid individuals and found that *the sex* in these individuals *was determined by a balance between the number of X-chromosomes and the number of haploid sets of autosomes. The Y-chromosome contains no sex-determining genes.* An individual with *X*-chromosomes and sets of autosomes in the proportion 1 : 1 will be a female; if the proportion is 1 : 2 the animal is a male, but if it is between 1 : 1 and 1 : 2 (as, e. g., 1 : 1,5, in the triploid fly with only 2 *X*-chromosomes) the individual will be an intersex. BRIDGES and his collaborators have (cf. BRIDGES, 1939) proved this theory in respect of triploid, tetraploid and aneuploid animals from different lines. According to BRIDGES, the sex-determining genes are located in the autosomes as well as in the *X*-chromosome. *In Drosophila the majority of the male-determining genes are to be found in the autosomes, when the X-chromosome contains female-determining genes.* That more than one female-determining gene must be found in the *X*-chromosome of *Drosophila* has been demonstrated by DOBZHANSKY and SCHULTZ (1934), who studied the effects of different fragments of the *X*-chromosome on the sex determination of the animal.

Only a few dioecious plants have been investigated in order to establish the location of their sex-determining genes. The first studies of this subject in the plant kingdom were made on *Rumex Acetosa* by the Japanese research-workers ONO (1935, and elsewhere), YAMAMOTO (1938, and elsewhere), and TAKENAKA (1938, and elsewhere). They examined mainly triploid and tetraploid natural intersexes and their

progeny. The conclusion made by these workers was that *the sex determination in Rumex Acetosa is in full accordance with the theory of* BRIDGES. According to YAMAMOTO (1938), two of the autosome pairs bear female-determining genes, three pairs are strongly male-determining and one pair bears a weak male determinant. The *X*-chromosome is strongly female-determining, but the *Y*-chromosomes seem to be inert as far as regards sex-determining genes.

The sex mechanism met with in Drosophila and Rumex Acetosa works against polyploidy, as no pure males will be produced in the polyploid forms. MULLER (1925) explained the absence of polyploidy in the animal kingdom (and within the dioecious plants) as a result of this fact. However, some of the dioecious plants and animals with manifestly polyploid chromosome numbers showed conditions which contradicted his hypothesis.

b. *Melandrium.* — In 1938, WESTERGAARD (1938 a) published a preliminary account of experiments on polyploid plants of *Melandrium album.* His experiments with heat-treatments on this dioecious plant succeeded in tetraploid individuals of both sexes, as indeed might be expected if the male and female individuals of the *Drosophila—Rumex Acetosa* type were doubled. WESTERGAARD cross-pollinated these individuals and gave the results in detail in another paper (1940). *The plants in the F_1 generation turned out to be dioecious contrary to the expected results, provided the same mechanism of sex determination as found in Drosophila was met with in Melandrium.* The conclusion drawn by WESTERGAARD on the basis of his results was that »*the sex in Melandrium male plants is determined by a balance between a very strong male-determining element in Y and a female-determining element in the autosomes and in X.*» This element in the *Y*-chromosome consists, according to WESTERGAARD, of a very few epistatic genes. His results have been confirmed by WARMKE and BLAKESLEE (1939, 1940) and ONO (1939, 1940 a) for *M. album* and by D. LÖVE (1940, 1942 a, and unpubl.) for *M. rubrum.* WESTERGAARD's conclusion as to the occurrence of female-determining genes in the autosomes was not based on his experimental results published in 1940 (cf. KUHN, 1942), but, according to unpublished results by D. LÖVE, who has studied different kinds of intersexual individuals and their progeny, *both female- and male*-determining genes may be found in the autosomes. The diploid hermaphroditic types with one *Y*- and two *X*-chromosomes obtained by WARMKE (cited from BLAKESLEE, 1941, p. 134) from triploids after a 4x ♀ × 2x ♂ cross, are also to be regarded as an indication of *female-*

determinants in the autosomes. According to WESTERGAARD (in lit.), a tetraploid *XXXX*-intersex is met with in the progeny he obtained from the experimental tetraploids, which likewise indicates the occurrence of *male*-determinants in the autosomes. The main difference between the mechanism of sex determination observed in *Drosophila—Rumex Acetosa* and *Melandrium* is that *in the Drosophila type the Y-chromosome is inert as regards sex-determining genes, and the autosomes bear chiefly male- and a few female-determining elements*, but *in the Melandrium type the Y-chromosome contains strong male elements and the X-chromosome is mainly female-determining. In the autosomes both male- and female-determining genes are found in a not yet known proportion.* This strong *Y*-mechanism makes even tetraploid forms of this species dioecious. Such a tetraploid dioecious form of *M. rubrum* has been reported from nature by D. LÖVE (1942 a).

c. *Achnida.* — Almost simultaneously MURRAY (1940 a, b) showed that the sex determination in the Amaranthaceae may be demonstrated with the aid of almost the same rule as in *Melandrium*. In the dioecious species *Achnida tamariscina* MURRAY was able to demonstrate that, although no sex chromosomes were detectable, the male is heterogamous with a genetical *XY*-mechanism. After doubling with colchicine it was found that the *XXXY* plants are males which breed true and give the sex ratio 1 : 1 if crossed with a tetraploid female (*XXXX*). As the *XXXY* plants are males, MURRAY concluded that the *Y*-chromosome probably carries the male-determining factors in *Achnida* and that the female-determining elements are probably located in the *X*-chromosome.

In a previous publication the present writer (LÖVE, 1941 b) gave a preliminary account of the sex determination in *R.* sg. *Acetosella*. He then showed that the dioecism of the polyploid species must be the result of strong male elements in the *Y*-chromosome, and that, contrary to *R. Acetosa*, the sex determination of *R.* sg. *Acetosella* must belong to the same type as in *Melandrium*. This suggestion has proved to be correct, as will be shown in the following pages.

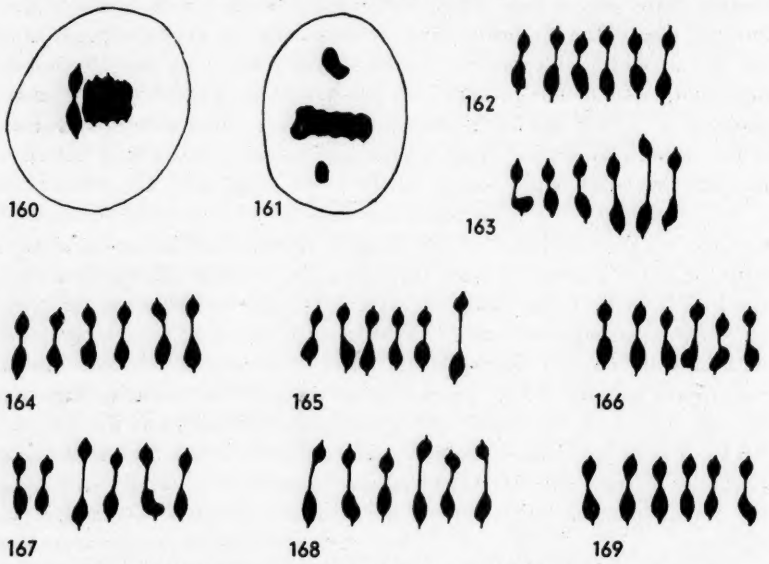
2. SEX DETERMINATION IN RUMEX SG. ACETOSELLA.

A. THE SEX CHROMOSOMES OF RUMEX ANGIOCARPUS.

a. *Evidence from meiosis.* — The unequal pair of sex chromosomes met with in all the higher plants in which sex chromosomes have been detected is generally studied at metaphase-I, where it is

found as a rod-bivalent (or a chain) (cf. DARLINGTON, 1937; SHARP, 1934).

The studies made by the present writer on the diploid species *R. angiocarpus* revealed that *at metaphase-I in the male plants a distinct pair of heterochromosomes was found*, and in almost all the cells studied it was an easy task to see them, as they are very often localised at one side of the plate (Fig. 160). At the prophase stages the sex chromosomes



Figs. 160—169. Sex chromosomes at meiosis in *R. angiocarpus*. — Fig. 160, the *XY* bivalent beside the metaphase-I plate; Fig. 161, precocious separation of the sex chromosomes; Figs. 162—169, *XY*-bivalents from different cells of different plants. — Magnification × 2100.

were observed in some cells. In diplotene they show »heteropychnose», as they seem to contract somewhat earlier than the other chromosomes (cf. GEITLER, 1938; DARLINGTON, 1937), and at anaphase-I they are seen to separate earlier than the autosomes in some cells (Fig. 161). As mentioned above (p. 15), the sex chromosomes sometimes form an »accessory plate». The sex-linked heterochromosomes were closely studied at metaphase-I in a number of cells in all the male individuals cytologically investigated. Six pairs of these chromosomes were drawn separately from different cells in eight individuals and they are reproduced in Figs. 162—169. As no divisions were obtained in the

megaspore mother-cells studied, it was not quite clear from the studies of meiosis whether the smaller or the larger chromosome represented the *Y*-chromosome in *R. angiocarpus*. In the animal and plant kingdoms the *Y* has been found to be the smaller one in almost all the cases hitherto examined. In *Rumex Acetosa*, the total mass of the Y_1 and Y_2 is larger than that of the *X* (ONO, 1926, 1935; KIHARA and YAMAMOTO, 1931; LÖVE, unpubl.), and in *Melandrium* the *Y* is the larger one of the sex chromosomes (WARMKE and BLAKESLEE, 1939, 1940; ONO, 1939, 1940 a; WESTERGAARD, 1940 for *M. album*, D. LÖVE, 1940, 1942 c for *M. rubrum*). Very exact measurements of the sex chromosomes can, however, only be made at the mitotic metaphase, as there they are very constant as to form and size and the constrictions can be defined with certainty.

Figs. 170—177. Sex chromosomes at mitosis in *R. angiocarpus*. — Figs. 170—172, *X*-chromosomes from three females; Figs. 173—177, *X*- and *Y*-chromosomes from five males. — Magnification × 3370.

b. *Evidence from mitosis.* — When investigating the sex chromosomes in the somatic metaphase plates the author used the same methods as when studying the karyotypes of the plants. From the measurements it was quite certain that both the sex chromosomes of the female plants (*XX*) were only slightly longer than the longest pair of autosomes. The centromere of the *X*-chromosome is submedian, the arms being 1,4 and 1,6 micra respectively. The total length of the *X*-chromosome is thus 3,0 micra, but the two longest pairs of autosomes are 2,7 micra long in the karyotype P_1.

The heterochromosomes were easily detected in the somatic metaphase plates of the male plants. The *X*-chromosome was always about 3,0 micra long as in the female individuals, but the total length of its partner, the *Y*, was 4,4 micra. Thus, *the Y-chromosome is the longer one in R. angiocarpus*. The centromere of the *Y*-chromosome is submedian and the length of its two arms is 2,0 and 2,4 micra respectively. Some sex chromosomes from the root-tip cells of different female and male

individuals are shown in Figs. 170—177 (Figs. 170—172: females; Figs. 173—177: males).

As may be seen from the differences in length between the two arms of the *X*- and *Y*-chromosomes, they differ slightly in the position of the centromere. In *Melandrium* these differences are more evident, as the constriction of the *Y* is strictly median, whereas it is submedian in the *X*. It is suggested that the *X*- and *Y*-chromosomes very often differ in the position of the centromere, and when no heterochromosomes are detectable in the heterogamous sex at metaphase-I they might possibly be demonstrated in the somatic metaphase plates if the position of their centromeres is different. In favourable material with relatively long chromosomes the measurements of the mitotic chromosomes must afford a far clearer indication of the occurrence of heterochromosomes than a study of the meiosis can give.

B. THE SEX CHROMOSOMES OF RUMEX TENUIFOLIUS.

a. *The meiosis.* — If the tetraploid species *R. tenuifolius* is to be regarded as a polyploid of the diploid species with the same sex mechanism as is found in the *Melandrium* tetraploids, it is to be expected that the males of this species would have *XXXY* sex chromosomes when the females had *XXXX*. As the three *X*-chromosomes must be, originally

TABLE 6. *The associations of the sex chromosomes in R. tenuifolius.*

Plant No.	Association			n
	XX + *XY*	*XXXY*	*XXY* + *X*	
R 077 — 5	15	5	—	20
R 0108— 4	19	—	1	20
R 0109— 4	10	8	2	20
R 0110—42	19	—	1	20
R 0111—42	17	1	2	20
R 0188—42	17	1	2	20
R 0604—42	14	3	3	20
R 0605—42	8	1	1	10
R 0609—42	7	1	—	8
R 0610—42	15	2	3	20
R 0615—42	16	1	3	20
R 0829—42	20	—	—	20
R 0831—42	19	—	1	20
R 0834—42	17	—	3	20
R 0844—42	17	1	2	20
R 0847—42	15	3	2	20
Total:	245	27	26	298

at least, identical and the one end of the *Y* must be differential from both ends of the *X*'s, only associations with chain-quadrivalents or a rod *XY*-bivalent and a ring- or rod-*XX*-bivalent will be formed at meta-

Figs. 178—187. Associations of sex chromosomes at meiosis in *R. tenuifolius*. — Magnification × 2100.

phase-I, or a chain *XXY* with an *X* univalent, or a ring or chain of *XXX* and a *Y* univalent.

The configurations of the sex chromosomes were analysed in 298 cells in 16 different individuals. Some of the associations observed are shown in Figs. 178—187. The frequencies of the different associations are given in Table 6.

Figs. 188—192. Sex chromosomes at mitosis in *R. tenuifolius*. — Figs. 188—189, the four *X*-chromosomes from two females; Figs. 190—192, the *Y* and the three *X*-chromosomes from three males. — Magnification × 3370.

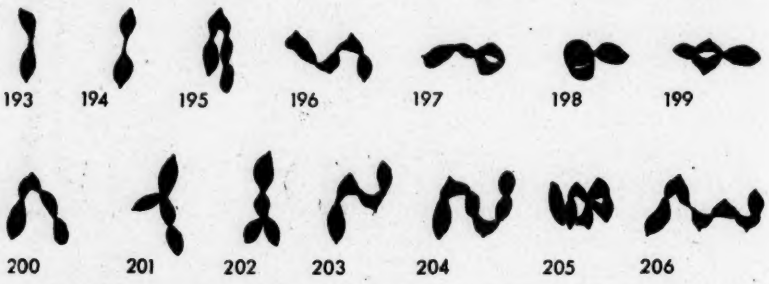
As shown in the table, the association $XY + XX$ is the most frequent, as it is found in 245 out of the 298 cells, or in about 82 % of the cases examined. The low frequency of higher associations must be due to more than mere chance. It is assumed to be the result of some minor changes in the homology of one of the *XX* pairs. *Two of the sex chromosomes are therefore being gradually differentiated on the way to becoming only a normal autosome pair*, as postulated by WESTERGAARD (1940).

b. *The somatic chromosomes.* — Some of the sex chromosomes from the root-tip cells of male and female individuals of *R. tenuifolius*

are depicted in Figs. 188—192. Figs. 188—189 are from two female plants, and Figs. 190—192 are from three male individuals. It is evident from these figures that the sex chromosomes morphologically resemble the *X* and *Y* from the diploid species. The measurements, moreover, gave almost the same results, i. e. the *X*'s were about 3,0 micra long and the *Y* about 4,4 to 4,5 micra long.

C. THE SEX CHROMOSOMES OF RUMEX ACETOSELLA S.STR.

As mentioned above, the hexaploid species of *R.* sg. *Acetosella* formed one of the most obscure cases in the previous investigations of the sex chromosomes in dioecious plants. According to the views of the present writer (LÖVE, 1941 b), it is to be regarded as an autopolyploid



Figs. 193—206. Associations of sex chromosomes at meiosis in *R. Acetosella* s.str. — Figs. 193—194, bivalent *XY*; Figs. 195—203, quadrivalent *XXXY*; Figs. 204—206, hexavalent *XXXXXY*. — Magnification × 2100.

(s. lat.) of the diploid species of the subgenus. As the diploid — and tetraploid — species were found to have an *XY* (and *XXXY*) mechanism of sex determination, it was expected that in the hexaploid species the sex chromosomes would be six in number, i. e. *XXXXXY* in the male plants with 2n = 42 chromosomes and *XXXXXX* in the females. The results of the studies of the meiosis in the tetraploid species showed, however, that the sex chromosomes must be expected as a bivalent *XY* pair or a chain of *XXXY* in most of the cells, but hexavalent sex chromosomes might be expected to exist only in a low frequency of cells if the same gradual differentiation of *X*-chromosomes to autosomes, as suggested in *R. tenuifolius*, is found in *R. Acetosella* s.str.

The associations of the sex chromosomes at metaphase-I were analysed in 169 cells in 14 different plants (cf. Table 7). As it was exceedingly difficult to distinguish the associations of the *X*-chromosomes when they were not associated with a *Y*-chromosome, only the

associations with a *Y*-chromosome were examined. When the sex chromosomes are found as a quadrivalent chain, one pair of *X*-chromosomes is to be met with as a bivalent or two univalents, but in the cells with an *XY*-bivalent the sex chromosomes may, theoretically, form two bivalents, or a trivalent and a univalent, or only univalents, or one bivalent and two univalents, or, in a few cases, a quadrivalent. In the few cases, however, in which these *X*-chromosomes were distinguished with a high degree of certainty in cells with an *XY*-bivalent, they occurred as *two bivalents.*

As will be seen in Table 7, the sex chromosomes were found as bivalents in 131 out of the 169 cells examined, or in about 78 % of the cells. In 31 cells, i. e. in about 18 %, a chain of *XXXY* was noted, and in only 7 cells — or about 4 % — was a hexavalent chain of the sex chromosomes observed. In three plants bivalent *XY* only was found. Some of the associations met with are depicted in Figs. 193—206. In no cell were trivalent nor pentavalent associations of *Y* and *X*'s observed.

These results show that *even in the hexaploid species some of the X-chromosomes seem to have gradually differentiated in the process of becoming autosomes.* This differentiation seems to have advanced somewhat further in one of the two extra pairs of *X*-chromosomes, to judge from the low frequency of the tetravalent association.

TABLE 7. *The associations of the sex chromosomes in R. Acetosella s.str.*

Plant No.	Association			n
	XY	*XXXY*	*XXXXXY*	
R 0133—39	14	—	—	14
R 0135—39	8	5	1	14
R 0138—39	9	2	—	11
R 0278—41	10	3	—	13
R 0628—42	7	3	1	11
R 0634—42	11	—	—	11
R 0638—42	10	4	1	15
R 0653—42	6	2	—	8
R 0656—42	7	2	2	11
R 0658—42	7	2	1	10
R 0794—42	7	2	—	9
R 0796—42	7	3	—	10
R 0839—41	20	—	—	20
R 0854—42	8	3	1	12
Total:	131	31	7	169

At mitosis it is not an easy task to determine the occurrence of the heterochromosomes in the hexaploid individuals. In a few plants, however, one large chromosome, possibly the *Y*-chromosome, was observed in some root-tip cells, but no exact measurements could be taken of this chromosome.

D. THE SEX CHROMOSOMES OF RUMEX GRAMINIFOLIUS.

As the meiotic behaviour of this octoploid species has not been examined, no description of the configurations of its sex chromosomes at meiosis can be given here. Theoretically, its sex chromosomes must be *XXXXXXXY* in the males and *XXXXXXXX* in the females with the exact octoploid chromosome number. They have, however, not been detected in the somatic metaphase plates as the chromosomes are almost invariably densely crowded on the plate.

E. THE SEX CHROMOSOMES OF R. ACETOSELLA S.STR. × R. TENUIFOLIUS HYBRIDS.

The sex chromosomes were studied in 23 cells of the two hybrid males cytologically examined. The results are given in Table 8. In 12 cells the sex chromosomes were found as one bivalent *XY* and the other *X*'s formed a trivalent in 8 of these cells and were found as a bivalent and a univalent in the 4 other cells. In 7 cells the sex chromosomes formed a trivalent *XXY* and a bivalent *XX*, in one cell a tetravalent *XXXY* and a univalent *X* were observed, and in 3 cells the sex chromosomes were found as a pentavalent *XXXXY*.

TABLE 8. *The associations of the sex chromosomes in R. Acetosella × tenuifolius.*

Plant No.	Association				n
	XY	*XXY*	*XXXY*	*XXXXY*	
R 0661	8	1	1	2	12
R 0664	4	6	—	1	11
Total:	12	7	1	3	23

Three of the *X*-chromosomes of these plants originated from *R. Acetosella*, but the *Y* and one *X* belonged to the male *R. tenuifolius*. Of course, it is not impossible that the bivalent *XY* with a trivalent *XXX* is formed by »autosyndesis», but the present writer suggests that in at least the majority of the cells the *Y* is paired to an *X*-chromosome from *R. Acetosella*. As the material is scanty, the proportions of the different associations do not give any definite indication of the possible difference between the *X*-chromosomes of the different species.

F. THE SEX DETERMINATION OF INTERSEXES.

So far, intersexes are only known within the hexaploid and octoploid species. In the octoploid species, according to TRAUTVETTER (1877), intersexual plants are fairly frequent in the herbaria, although the present writer has not observed any intersexual individual in the herbaria in Scandinavia. Intersexual plants are very infrequent in the hexaploid species. In the material obtained by the present writer there is only one single intersexual plant, i. e. *R 0138—39* from Frösön, and one intersexual plant has been reported by ONO (1930). According to ONO, this plant was a male with some intersexual flowers, and only its chromosome number was studied closely. It was $2n = 41$, but as ONO did not observe sex chromosomes in the species, he drew no conclusions as to the nature of the lost chromosome. The present writer, however, suggests that the lost chromosome was an autosome, as the loss of an *X*-chromosome cannot possibly result in intersexuality.

The plant studied by the present writer showed the exact hexaploid number $2n = 42$, and at meiosis the sex chromosomes formed a bivalent in 9 cells and a quadrivalent *XXXY* in 2 cells. It was a male plant with a few intersexual flowers, and many seeds were obtained from it after isolation. The progeny proved to be both males and females, but as no close examination has as yet been made of the cytology of these plants no definite results thereof will be given here.

The preliminary results of the enquiry into the intersexuality of *R. Acetosella* lead to the suggestion that it is due not to disturbances in the mechanism of the sex chromosomes but to an accumulation of female-determining genes in the autosomes. This is, in the view of the present writer, the most probable explanation of the occurrence of intersexuality in the hexaploid and octoploid species, and will be more closely examined and discussed in a later paper. It is based on the hypothesis that female- and male-determining elements are also found in the autosomes of *R.* sg. *Acetosella.*

G. CONCLUSIONS.

The following inferences may be drawn from the above-mentioned results: *The sex determination in R. sg. Acetosella is fundamentally different from the mechanism met with in R. sg. Acetosa sect. Euacetosae*, since the male factors are located in the autosomes in the latter-mentioned group, but they are located mainly in the *Y*-chromosome in the species of sg. *Acetosella.* The epistatic male factors found in the *Y*-chromosome of the *Acetosella* group are strong enough to dominate the female elements in the *X*-chromosome and the autosomes, even in the hexaploid and octoploid species, as they are both dioecious. According to this, *the sex mechanism belongs to the same type of localization of sex-determining genes as found in Melandrium* (*and Achnida*?),

but the epistatic male determinants seem to be considerably stronger than in Melandrium. The X-chromosomes of the polyploid species seem to be on the way to a gradual differentiation into autosomes, as an *XY*-bivalent is the most frequent association of the sex chromosomes in the tetraploid and hexaploid plants examined. Later on some remarks will be made on the significance of these results as affecting the differentiation of the two dioecious subgenera of *Rumex* in particular and the evolution of sex chromosomes and sex mechanism in general.

IX. SEX RATIO.

It is a well-known phenomenon that in the majority of dioecious plants and animals the sex ratio is, on an average, 50 % males : 50 % females, or 1 : 1. In a few animals, as well as in some dioecious plants (cf. WINGE, 1937; CORRENS, 1928), deviations from this ideal distribution have been observed. In the cases hitherto reported the heterogametic sex seems to be in the majority in animals, but in the minority in plants. This phenomenon must be due to some physiological or genetical phenomena linked to the germ cells as the theoretical sex ratio 1 : 1 is always met with at metaphase-anaphase in plants and animals with an *XY*-mechanism of sex determination. A detailed account of the different hypotheses relating to the phenomenon adduced by various students is given by CORRENS (1928).

In the genus *Rumex* the species of section *Euacetosae* show a distinct preponderance of females (cf. CORRENS, 1928; STECKHAN, 1937; LÖVE, 1940 b, 1942 a), and according to OHGA (cited from SINOTO, 1929, p. 177) this is also the case in *Rumex Acetosella*.

According to the cytological explanation of the sex mechanism in *R.* sg. *Acetosella*, gones with one *Y*-chromosome are male-»determining» in all the four species. In all the species such gones will be found in 50 % of the pollen grains. If the two kinds of pollen show the same percentage of germination and there is no competition between pollen tubes with *X* and *Y*, on an average 50 % of the eggs fertilized will be males with a *Y*-chromosome, the other 50 % being females with only *X*-chromosomes. If no differences occur in germination percentage, nor in the death-rate of males and females, nor a high frequency of agamospermous seed formation, the mature male and female plants will be found in about 1 : 1 ratio in nature and by experiment.

The proportions of the sexes in my experimental material as well as from some natural localities are given in Table 9.

The numbers in Table 9 are, of course, too low to give an absolute value of the sex ratio of these species, but the tetraploid species with the highest number of observations shows almost the exact ratio 1 : 1, and no statistical significance is attached to a deviation from this ratio in the diploid and hexaploid species. Thus, the present writer assumes that *the sex ratio of these species will show practically no deviations from the 1 : 1 proportion in nature*, except for populations with a high frequency of agamospermy, where the female plants must be in the majority (cf. MURBECK in NORDSTEDT, 1907).

TABLE 9. *The sex ratio in Rumex sg. Acetosella.*

Species	Origin	Males	Females	Not flowering
R. angiocarpus	Lisbon (experim.)	31	26	18
R. tenuifolius	Rickomberga (exp.) I. ..	6	17	—
»	» » II. ..	15	9	—
»	Budapest »	8	8	—
»	Liège »	39	25	—
»	Cluj »	2	3	—
»	Moscow »	5	5	—
»	Åhus (in nature)	343	350	—
	Total:	418	417	—
R. Acetosella s.str.	Fossvogur, Iceland (exp.)	11	11	—
»	Reykjavík, » »	20	20	—
»	*R 0136* × *R 081* »	5	6	—
»	Helsingfors (experim.)..	16	9	—
»	Frösön » ..	20	20	—
»	Frösön (in nature)	7	2	—
	Total:	79	68	—

X. CHROMOSOME NUMBER AND MORPHOLOGY.

1. PREMISES.

The morphology of the species of *R.* sg. *Acetosella* has been examined from two different points of view, the taxonomical and the genetical, in combination. The taxonomical studies include mainly the gross morphology of the different species and forms. They are carried out on the basis of herbarium studies and genetical experiments; their results will be closely examined in another paper (LÖVE, unpubl.). The results mentioned in this chapter include only some of the genetical comparative studies made in order to elucidate some of the minor morphological differences occurring between the polyploid species. At the same time they may bring some new data to bear on the effect of polyploidy on different morphological details.

It has been shown by numerous authors (cf. MÜNTZING, 1936; WETTSTEIN, 1940; KOSTOFF, 1941) that polyploid plants differ from their diploid relatives in a great many quantitative characteristics, which for the most part seem to be caused directly by the higher number of chromosomes, which is almost invariably accompanied by a larger size of cell. It has sometimes been observed, however, that the size of the different organs of the individual do not increase proportionally

to the increase in the chromosome number. This fact must be due to a unitary decrease in the number of cells in the organ (cf. MÜNTZING, 1936).

In *R.* sg. *Acetosella* it is of considerable interest to control whether the differences between the four species are caused directly by the degree of polyploidy and also whether all the organs react in the same way to the polyploidy. The height of the plants do not seem to be correlated with the polyploidy, according to an examination of experimental and herbarium material of all the species from different localities. Most of the minor differences between the species seem, however, to be caused directly by the polyploidy. All the best taxonomical characteristics of the species — the differences in the floral parts — seem to be a result of the differences in chromosome number, as will be seen in the following pages concerning the size of the female flowers, anthers and fruits. As to some of the characteristics which seem at first glance to be of the qualitative kind, they, too, may often be regarded as being quantitative, or caused directly by the polyploidy. For instance, the »qualitative» difference »angiocarpus» contra »gymnocarpus» is a difference in the relative length of the inner perigon and the achene. In the diploid species the achene and the inner perigon are of about the same length, and the inner perigon includes the entire fruit. They are also grown together. In the tetraploid species both the size of the fruit and the inner perigon have increased, but the length of the inner perigon has increased somewhat more than the length of the fruit. It is, therefore, open at the top. In the hexaploid species the relative differences in size between the inner perigon and the achene have increased somewhat more. Here the inner perigon is fully open. The inner perigon of the octoploid species is almost twice as long as the ripe fruit and includes the achene only in the manner known from the other subgenera of *Rumex*. Thus the »qualitative» character »angiocarpus» contra »gymnocarpus» is really only a quantitative character. This is evident from Figs. 210—213.

One difference met with in the writer's material of the four species is possibly caused by some qualitative factors. The number of layers of pallisad cells in the leaves was studied in different material of all the species. In *R. angiocarpus* and *R. Acetosella* s.str. only *one* layer of pallisad cells was observed, but in *R. tenuifolius* and *R. graminifolius* there were *two* layers. The material is, however, too scanty, as only a few forms of the different species have so far been studied in this respect.

2. THE INVESTIGATIONS.

A. THE SIZE OF THE NUCLEUS.

The size of the nucleus was studied in the meristeme cells of the roots in the parts with the highest frequency of divisions. Only cells in the resting stage were examined. The results are given in Table 10.

TABLE 10. *The size of the nucleus in somatic resting stages.*

Species	Diameter in μ	Volume in μ^3	Ratio (Volume)
R. angiocarpus (2x) ...	$6{,}02 \pm 0{,}14$	$114{,}24\,\mu^3$	100
R. tenuifolius (4x)	$7{,}38 \pm 0{,}10$	$210{,}47\,\mu^3$	184
R. Acetosella s.str. (6x)	$8{,}59 \pm 0{,}19$	$331{,}89\,\mu^3$	291
R. graminifolius (8x) ..	$9{,}98 \pm 0{,}25$	$520{,}49\,\mu^3$	456

As the table shows, there is *a clear increase in size from the diploid to the octoploid state.*

The author was not able to count the maximum number of nucleoli in the hexaploid and octoploid species, but in the diploid never more than two were observed, and in the tetraploid never more than four. These numbers coincide with the number of SAT-chromosomes found in the somatic metaphases. In the resting stage only one large nucleolus was observed in each cell, but it showed clear differences in size in the different polyploid species, as well as in male and female plants. In male plants it was always somewhat larger than in the females, possibly owing to some fusion with bodies of heterochromatin? (Cf. DARLINGTON and LA COUR, 1942).

B. THE SIZE OF THE CELLS.

An almost general characteristic of polyploid plants in relation to their diploid relatives is the increase in cell size correlated with the increase in the chromosome number. References to discussions on this well-known phenomenon are to be found in MÜNTZING (1936), WETTSTEIN (1940), KOSTOFF (1941), and GYÖRFFY (1941 b).

a. *Size of stomata.* — The stomata were measured in plants at the same stage of development, cultivated in pots. The conditions were absolutely the same. The measurements are therefore comparable, although the climatic conditions may have been somewhat unsuitable to the diploid and octoploid plants.

The measurements were taken on the central parts of some leaves *in vivo* in a sugar solution coloured with some drops of neutral red. The results obtained are given in Table 11.

As may be seen in Table 11, the length of stomata increases from the diploid to the tetraploid state and from the tetraploid to the hexa-

ploid state. The difference is statistically significant as D/m = 9,2 for *R. angiocarpus* : *R. tenuifolius* and D/m = 9,3 for *R. tenuifolius* : *R. Acetosella* s.str. From the hexaploid to the octoploid state, however, a *diminution* in the length of stomata is calculated. The negative difference between *R. Acetosella* and *R. graminifolius* is statistically significant, giving D/m = 17,2. The decrease met with in *R. graminifolius* gives almost the same length of stomata as that calculated for *R. tenuifolius* (D/m = 1,4). This phenomenon is most probably due to the fact that the measurements of the stomata of *R. graminifolius* were taken on one relatively weak plant. It is, however, not quite out of the question that such a decrease really could be found, although it is contradicted by the results of the measurements of pollen grains (see below). A decrease in the stomata length is met with, e. g., in the autopolyploid forms of *R. Acetosa*, where a decrease is observed from the 5x to the 6x state (YAMAMOTO, 1938).

b. *Pollen diameter.* — The diameter of the pollen grains was measured in some individuals of all the species in acetocarmine-glycerine. The pollen from *R. graminifolius* originated from herbarium material, but all the other plants were cultivated in the experimental field.

The average size of the pollen increases very significantly as the chromosome number increases from 14 to 56. The ratio is 100 : 112 : 121 : 137.

In order to estimate the ratio of increase in volume, this was calculated from the average pollen diameter. The ratio of the volumes was: 2x : 4x : 6x : 8x = 100 : 136 : 171 : 251. Thus, the pollen grains increase about twice as much from the diploid to the octoploid state as from the diploid to the hexaploid, and also about twice as much from the diploid to the hexaploid as to the tetraploid.

The pollen size shows a very clear positive correlation to an increase in chromosome number in all the species studied. The differences in size are so distinct that they may be of great help in determining the systematic position of herbarium material. As the pollen of herbarium material is also found to decrease somewhat in volume with age (unpubl. data) only relatively young material from the herbaria is comparable. The definite increase in the size of the pollen grains is also noted in the autopolyploid plants of *R. Acetosa* (YAMAMOTO, 1938), where, however, it may also be due to meiotic irregularities. On the other hand, the different polyploid species of the subgenus *Lapathum* show no differences in pollen size, according to KIHARA and ONO (1926).

TABLE 11. *The length of stomata in R. sg. Acetosella.*

Units:	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	n	M ± m	Ratio
R. ang.	1	2	6	8	13	16	8	1	2	3	—	—	—	—	—	—	—	—	—	—	60	16,46 ± 0,24	100
R. ten.	—	—	—	—	—	6	6	6	8	7	8	4	3	2	—	—	—	—	—	—	50	20,42 ± 0,36	124
R. Acet.	—	—	—	—	—	—	—	2	4	2	9	8	4	6	6	1	5	1	—	2	50	24,04 ± 0,16	146
R. gram.	—	—	—	1	2	1	2	4	4	14	12	7	3	—	—	—	—	—	—	—	50	20,94 ± 0,09	127

TABLE 12. *Pollen diameter in R. sg. Acetosella.*

Units:	6	7	8	9	10	11	12	13	n	M ± m	Ratio	Volume	Ratio (Vol.)
R. angiocarpus ..	2	51	150	39	1	—	—	—	243	7,94 ± 0,04	100	268,71	100
R. tenuifolius	—	12	200	750	85	—	—	—	1047	8,86 ± 0,03	112	364,18	136
R. Acetosella	—	—	83	431	750	48	—	—	1312	9,58 ± 0,02	121	460,38	171
R. graminifolius..	—	—	—	8	64	150	41	2	260	10,88 ± 0,05	137	674,38	251

TABLE 13. *The length of the female flowers in R. sg. Acetosella.*

Units:	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	n	M ± m	Ratio
R. ang.	1	11	5	4	7	2	4	3	3	—	—	—	—	—	—	—	—	—	—	—	—	30	22,33 ± 0,39	100
R. ten.	—	1	1	2	5	3	1	5	6	2	1	1	—	—	2	—	—	—	—	—	—	30	24,67 ± 0,56	110
R. Acet.	—	—	—	—	—	—	—	—	—	—	1	3	1	2	2	4	5	4	6	1	1	30	32,47 ± 0,47	145

TABLE 14. *The length of the anthers in R. sg. Acetosella.*

Units:	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	n.	M ± m	Ratio
R. angioc.	2	2	7	7	6	3	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	30	34,17 ± 0,35	100
R. tenuif.	—	—	—	—	2	—	1	2	2	2	1	4	1	2	4	3	2	2	1	1	—	—	—	30	42,93 ± 0,68	126
R. Acetos.	—	—	—	—	—	—	—	—	—	—	—	1	2	2	3	4	4	4	4	2	1	2	1	30	47,30 ± 0,50	139

It is evident from the values given in Table 12 that the pollen diameter of all the four species studied forms a distinctly unimodal curve. This fact is of special interest as some investigators (cf. SINOTO, 1929) have supposed that the pollen of dioecious plants has a bimodal curve on account of the different mass of chromatin in grains with *X* and *Y*. This somewhat unintelligible suggestion was first used in order to explain the supposed competition between male- and female-determining pollen tubes in dioecious plants with an excess of females (cf. CORRENS, 1928). As the differences in chromatin mass between *X* and *Y* are rather small in relation to the mass of all the autosomes in the cells, this certainly not can account for any measureable differences in the size of the pollen grains. According to GREGUSS (1927) and others, a bimodal curve was found in *Melandrium*, but, as shown by D. LÖVE (1940) for *M. rubrum*, and by WESTERGAARD (1940) for

207 208 209

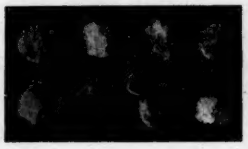
Figs. 207—209. Female flowers. — Fig. 207, from *R. angiocarpus*; Fig. 208, from *R. tenuifolius*; Fig. 209, from *R. Acetosella* s.str. — Magnification × 4,5. Photo: H. OLSSON, Svalöf.

M. album, this is not correct. According to the present writer (LÖVE, 1940 b), the pollen curve of *R. Acetosa, R. tenuifolius*, and *R. Acetosella* is distinctly unimodal, and the same has now been found in *R. angiocarpus* and *R. graminifolius*. The male-determining pollen grains in *R. Acetosa*, have, however, 8 chromosomes, the females only 7, which gives the ratio female : male = 100 : 114, or 14 % increase in the chromatin mass. As, moreover, this clear increase shows no influence on the mode of the curve, it must be assumed that *the pollen of dioecious plants will never show a bimodal curve of pollen size as a result of differences in the mass of chromatin in male- and female-determining pollen grains.*

C. THE SIZE OF THE FLOWERS.

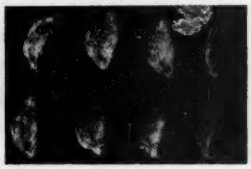
a. *The female flowers.* — The female flowers were collected from different individuals and the length of non-pollinated flowers was measured from the base to the top, the style not being included in the measurements. The results obtained are given in Table 13.

A clear correlation is found between the chromosome number and the size of the female flowers, the ratio 2x : 4x : 6x being 100 : 110 : 145. Unfortunately, the flowers from the octoploid species could not be included in the present calculations, but according to examinations made of herbarium material, the female flowers of *R. graminifolius* are distinctly larger than the flowers of the hexaploid species. The differences in the size of the female flowers of the three species studied are shown in Figs. 207—209.

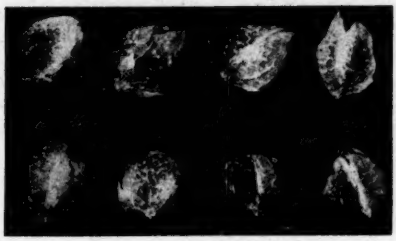


210

211



212



213

Figs. 210—213. Fruits with inner perigon leaves. — Fig. 210, from *R. angiocarpus*; Fig. 211, from *R. tenuifolius*; Fig. 212, from R. *Acetosella* s.str.; Fig. 213, from *R. graminifolius*. — Magnification × 5,5. — Photo: H. OLSSON, Svalöf.

b. *The anthers.* — In order to obtain a comparison between the male flowers some measurements were taken of the length of the anthers from different flowers of male plants. The results are given in Table 14.

The anthers also show a very clear increase in size correlated with an increase in chromosome number. The ratio 2x : 4x : 6x was 100 : 126 : 139. The anthers were also found to be larger in herbarium material of the octoploid than of the hexaploid species. Their length is quite a good taxonomical characteristic when determining male individuals of the different species.

D. THE SIZE OF THE FRUIT.

The size of the fruit shows a very clear increase from the tetraploid to the hexaploid state and from the hexaploid to the octoploid,

but the differences in size are rather small between the diploid and the tetraploid. The 1000-grain weight of the achenes together with the inner perigons was found to be: for *R. angiocarpus* = 261 g, for *R. tenuifolius* = 389 g, for *R. Acetosella* s.str. = 538 g, and for *R. graminifolius* = 1173 g. The ratio 2x : 4x : 6x : 8x is 100 : 149 : 206 : 449. The fruits are depicted in Figs. 210—213, where the differences in size as well as in the relation between the achene and the inner perigon may be seen.

E. THE CHLOROPLASTS.

According to KOSTOFF (1938), KOSTOFF and ORLOV (1938) and others, no statistically certain differences in the size of the chloroplasts are found between diploid and polyploid plants of the same group. The present writer took some measurements of the chloroplasts in the pallisad layers of three of the species of sg. *Acetosella*, with the results given in Table 15.

TABLE 15. *The size of the chloroplasts in R. sg. Acetosella (1 unit = 0,33 μ).*

Units:	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	n	M ± m
R. angioc.	1	1	2	1	2	4	4	9	5	10	4	4	2	1	—	—	50	27,48 ± 0,40
R. tenuif.	1	2	1	4	4	3	6	7	3	5	4	5	2	1	1	—	50	27,08 ± 0,46
R. Acetos.	—	1	2	1	3	3	7	4	5	7	7	4	2	3	—	1	50	28,00 ± 0,44

As the table shows, no differences in the size of the chloroplasts in *R.* sg. *Acetosella* are statistically demonstrable in this material. This lack of correlation between the chloroplast size and chromosome number is assumed to be due to their genetic independence (cf. KOSTOFF, 1941).

XI. CHROMOSOME NUMBER AND PHYSIOLOGY.

1. INTRODUCTION.

Physiological differences had already been presumed by the first students of this subject to be the main causes of the differences in the distribution of diploids and polyploids. It is, however, becoming apparent from a number of researches on diploids and their experimentally produced autotetraploids that the same general tendency to an increase as might have been expected from the morphological examinations is not always met with in the physiological and chemical conditions of the plants. This may be seen in the reviews given by GYÖRFFY (1941 b), MÜNTZING (1936), PIRSCHLE (1942 a, b, c), RANDOLPH (1941),

and STRAUB (1940, 1941). In order to obtain more comparable results, almost all the previous workers have used diploids and experimentally produced tetraploids for the investigations. As, however, it is of major importance to control the physiological and chemical differences in polyploid series, which have for a long time been differentiated by natural selection (cf. GYÖRFFY, 1941 b; SCHLÖSSER, 1940), the present writer has made some studies of the polyploid series of the *Acetosella* group. Some of the results obtained have previously been published (LÖVE, 1941 b).

2. METHODS.

The material used for the investigations was cultivated in the experimental field at Svalöf, except for *R. graminifolius*. Only plants at the same stage of development were used.

The *osmotic pressure* was obtained from leaf-cells according to the plasmometrical method in solutions of saccharose (0,10—1,5 mol.). As this method is not as exact as the cryoscopic method used by GYÖRFFY (1941 a, b) and SCHLÖSSER (1936), a large number of samples had to be analysed each time. The samples were taken in the morning on the rosette leaves of the plants.

The *water content* was determined each time from 3×1 g of leaves which were put in china bowls, which were then filled with ether. When the ether had evaporated, the samples were dried at 70° C over night and then weighed. The *ash content* was obtained after burning in an electric kiln for one hour at 700—800° C.

The *content of oxalic acid* was determined in 2×1 g of leaves, which were placed in china bowls in 25 cm^3 of 10 % hydrochloric acid. The samples were heated in a water-bath for 20 minutes and then removed and left over night. The hydrochloric acid was almost neutralized by 7 cm^3 of 37 % NaOH, before the samples were transferred over a flush of small quantities of water to a cradle-extraction-apparatus (cf. WIDMARK and AHLDIN, 1933). The oxalic acid was absorbed in 20 cm^3 of 5 % calciumchlorid with a vestige of sodium acetate. After extracting for 48 hours the calcium oxalate was defiltered and its quantity was determined by titration by permanganate.

The *content of ascorbic acid* was determined according to the following method: 3×1 g of leaves were torn up with sand and 25 cm^3 of 2 % metaphosphoric acid, which was put in the bowl in small quantities. When a minute suspension was obtained, it had to be clear before 10 cm^3 were taken into a pipette and titrated with 0,001-n 2,6-dichlorphenolindophenol. The values obtained show the content of reducing ascorbic acid.

For determining p_H 2×1 g of leaves were torn up in a china bowl together with water free from carbon dioxide. The water was poured into the bowl in small quantities. The suspension was mixed with kinhydron and the p_H was measured with the VEIBEL-electrod (0,01-n HCl + 0,09-n KCl).

Only the mean values ar given below. They are the results from analyses of a number of plants from different localities and in the same stage of development.

3. THE INVESTIGATIONS.

A. RATE OF GROWTH.

Numerous investigators (cf. MÜNTZING, 1936; STRAUB, 1941) have observed that polyploid »races» generally show a slower rate of growth than the diploids, and the same fact has been found in almost all strictly autopolyploid plants produced in experiments (cf. KOSTOFF, 1941; RANDOLPH, 1941; STRAUB, 1941).

The rate of growth of *R.* sg. *Acetosella* follows the same general rule as the plants studied previously. In my experiments the plants of the diploid species have flowered about 5—6 months after germination of the seeds. About 10 % of the tetraploid species have flowered during the first summer, about 5—6 months after germination, but 90 % of the plants did not flower until the second summer. The hexaploid species never flowered before the second year after germination, and a few plants (about 5 %) formed only a rosette the first two years. The octoploid plants were only cultivated in pots, and they never flowered during my experiments. For a year and a half, however, they only formed 5—6 rosette leaves.

The germination power of the seeds was very different in the four species. Seeds from the diploid species were found to germinate in about one week (5—8 days), the tetraploid seeds germinated in 9—12 days, and the hexaploid seeds germinated in 12—16 days. The seeds of the octoploid species, however, needed about one month (25—36 days) to germinate.

The slower development of the polyploid plants seems to be correlated with a larger vegetative development. The diploid plants form shoots almost solely from the top of the main root, as is the case, e. g., in *R. Acetosa* (cf. KORSMO, 1926). The tetraploid species shows a low frequency of vegetative root-shoots. In the hexaploid species such shoots are found in great numbers in every plant, and the octoploid plants seem to form root-shoots in a much higher frequency than the other species. In my material of the last-mentioned species root-shoots were found in the pots already a short time after germination.

B. OSMOTIC PRESSURE.

From observations on polyploids in nature it was apparent that their hardiness was generally greater than that of their diploid relatives. Accordingly, the osmotic pressure, which may greatly influence the winter-hardiness, was expected to be higher in the polyploids. Accord-

ing to BECKER (1931), however, osmotic pressure in autopolyploid mosses is inversely proportional to the chromosome number, and the same was found by SCHLÖSSER (1936) in regard to tomatoes and turnips, and by GREIS (1940) in *Hordeum*. If the part played by osmotic pressure in the hardiness of plants really was as important as expected, these first results seemed to militate against the hypothesis. Very similar results were obtained by GYÖRFFY (1941 a, b), who studied diploids and experimentally produced autotetraploids of *Capsicum, Lycopersicum, Hyoscyamus, Petunia, Antirrhinum*, and *Epilobium*. He examined, however, the osmotic pressure at different periods of development and found a wide variation in osmotic pressure in both diploids and polyploids. The tetraploids showed a considerably greater increase of osmotic pressure in a »dry chamber» than the diploids. GYÖRFFY (1941 a) concluded that »niedrigere osmotische Werte der 4n das Primäre sind» and that »die osmotischen Werte der Tetraploiden durch die Aussenbedingungen stärker abgeändert werden als die Diploiden». This phenomenon seems to be correlated with the tetraploids' higher power of resistance at least to drought (GYÖRFFY, 1941, a, b).

The osmotic pressure in *R.* sg. *Acetosella* was studied in the leaves of all the species at the same stage of development. All the samples were examined during the morning hours. The results are given in Table 16.

TABLE 16. *The osmotic pressure in rosette leaves of R. sg. Acetosella (in mol. saccharose).*

	Normal moisture		Dry, warm weather
	The youngest leaf	The 3rd leaf from the top	The youngest leaf
$2x$:	0,210 ± 0,013	0,220 ± 0,010	0,227 ± 0,006
$4x$:	0,267 ± 0,009	0,300 ± 0,012	0,317 ± 0,018
$6x$:	0,333 ± 0,014	0,383 ± 0,008	0,405 ± 0,021
$8x$:	—	0,425 ± 0,017	—

As the table shows, *the osmotic pressure of each species is higher in the older leaves than in the youngest one, and there is also an increase in dry, warm weather.* The differences are statistically significant. *A very definite increase is also found from the diploid to the tetraploid, hexaploid and octoploid state,* contrary to all the results relating to autopolyploids reported by previous investigators.

According to SPRECHER (1913), a higher osmotic pressure is observed

in the males than in the females of *R. Acetosa* and *Cannabis*, and LOEHWING (1933) reported the same from *Cannabis* and *Spinacia*. In my material of 2x, 4x and 6x species of *R.* sg. *Acetosella* no distinct differences were observed between the osmotic pressure of males and females.

Attention may be focused on the winter-hardiness of *R.* sg. *Acetosella* in connection with the osmotic pressure. A large number of plants have been cultivated in the experimental field at Svalöf during the years 1939—1942. The winters have been extremely cold the last two years. Scarcely 5 % of the diploid species were found to survive the first winter, but during the last two winters all the diploids died out. The tetraploids were much hardier, as almost 90 % of the plants survived even the winter 1941—42. The hexaploid plants survived all three winters. The extreme dry weather in the summer of 1941 made a definitely larger selection between the tetraploids than the hexaploids, but as the diploid plants were occasionally watered during the dry period, their resistance to drought could not be studied.

C. CONTENT OF WATER, ASH AND OXALIC ACID.

The water content of diploid and autotetraploid plants has been determined by several investigators. Some have reported an increase, while others have found no significant differences or even a decrease. The same seems to apply also to the ash content. An increase in water content and a corresponding decrease in dry matter is reported by SCHLÖSSER (1940) and by PIRSCHLE (1942 a) in most of the plants studied by them, but a few species showed no appreciable differences between diploids and autotetraploids. A decrease in water content is found by NOGUTI, OKA and OTUBA (1940) in autotetraploid *Nicotiana Tabacum*.

The results from the analysis of water content in twelve different individuals of the diploid, tetraploid and hexaploid species of *R.* sg. *Acetosella* are given in Table 17. According to the results of the analysis of osmotic pressure, a decrease in water content was expected, as a higher osmotic pressure may be due to a decrease in the water content of the cell sap (cf. PIRSCHLE, 1942 b). It may, however, conceivably be controlled in some way by the contents of the cytoplasm itself, perhaps by some differences in the viscosity of the plasm.

The ash content was examined in the same twelve plants. As the table shows, it was found to *decrease* from 2x to 4x and 6x, if calculated in per cent. of fresh matter, but a decrease was found only from the

TABLE 17. *The content of water, ash and oxalic acid in R. sg. Acetosella.*

	2x	4x	6x
Water content:			
% of fresh matter:	83,27 ± 0,93	82,43 ± 0,78	84,01 ± 1,02
Ash content:			
% of fresh matter:	1,560 ± 0,038	1,397 ± 0,026	1,292 ± 0,029
% of dry matter:	9,330 ± 0,126	8,715 ± 0,092	8,753 ± 0,098
Oxalic acid:			
% of fresh matter:	2,130 ± 0,034	1,738 ± 0,029	1,419 ± 0,041
% of dry matter:	13,05 ± 0,11	11,10 ± 0,13	10,64 ± 0,09

diploid to the tetraploid state if calculated in per cent. of dry matter.

The content of oxalic acid is a characteristic feature of the genus *Rumex*. As Table 17 shows, a statistically significant decrease of oxalic acid content is found to be correlated with an increase in the chromosome number.

According to a number of students (cf. STANFIELD, 1937; LOEHWING, 1938), some differences in the content of dry matter and ash in males and females have been reported from different dioecious plants. In both these conditions, however, tendencies to differences between the two sexes arise without any general rule. In the content of dry matter the present writer was not able to obtain any differences between the sexes, but in ash content some differences were observed. The *females* of *R. tenuifolius* showed a significantly *higher* percentage of ash content than the males, but the *males* of *R. Acetosella* s.str. showed a somewhat higher percentage of ash content than the females. The same differences appeared in the content of oxalic acid, the *females* of *R. tenuifolius* and the *males* of *R. Acetosella* s.str. showing the higher percentages.

D. CONTENT OF ASCORBIC ACID.

In an analysis of the vitamin C content of apples (CRANE and ZILVA, 1931; cf. also NILSSON-EHLE, 1938), tomatoes (SANSOME and ZILVA, 1936), *Rosa* (PYKE and MELVILLE, 1942, cited from DARLINGTON, 1942), and some other plants, results showing an increase from the diploid to the tetraploid (or triploid) state have been obtained. According to JOHANSSON (1939), however, the polyploid apples do not always contain more vitamin C than the diploid ones, and GYÖRFFY (1941 b) was not able to find any correlation between polyploidy and content

of vitamin C in some varieties of *Capsicum annuum* and *Lycopersicum*. He concluded, therefore, that »apparently there is no direct connection between the ascorbic acid content and number of chromosome sets, at least not in every autopolyploid».

Some individuals of the 2x, 4x and 6x species of sg. *Acetosella* were analysed for the content of ascorbic acid in the leaves. The results are given in Table 18.

TABLE 18. *The content of ascorbic acid in R. sg. Acetosella (mg/100 g, mean values).*

	2x		4x		6x	
	♀	♂	♀	♂	♀	♂
First sample (3 × 1 g)	85	87	69	78	62	68
Second » (»)	—	86	79	74	53	59
Third » (»)	—	—	73	76	—	68
Mean:	86,0		74,8		62,0	

All the samples were taken from the rosette leaves of flowering plants on the 5th—21st June in dry weather. The different samples were taken from different individuals.

Although the variation in vitamin C content seems to be fairly wide in all the species, *a distinct decrease is found from the diploid to the hexaploid condition.* This lends still further support to GYÖRFFY's conclusion mentioned above, as the content of vitamin C here is found to be inversely proportional to the number of chromosomes.

The males seem to have somewhat more vitamin C than the females. The differences are, however, not statistically significant.

E. P_H OF SAP.

The p_H of sap was analysed in some individuals of both sexes. It was found to be: in the diploid species $= 3{,}15 \pm 0{,}08$, in the tetraploid species $= 3{,}52 \pm 0{,}06$, and in the hexaploid species $= 3{,}68 \pm 0{,}05$. These values show *a distinct increase correlated with an increase in chromosome number.* In all the species the values for the males were somewhat lower than for the females. No statistical significance, however, attaches to this fact as between the sexes.

F. CONTENT OF CHLOROPHYLL.

The content of chlorophyll was studied in a number of individuals of the diploid, tetraploid and hexaploid species. The method used was as follows:

2 g of fresh leaves were collected from each plant and the leaf pigments were extracted in 10 cm^3 of 96 % alcohol for about 24 hours. Then 1 cm^3 of the extract was colorimetrically compared with a standard solution and diluted with alcohol until the two solutions showed the same intensity of colour. The quantity of the solution was carefully measured before and after dilution. The values given in Table 19 demonstrate to how many cm^3 a 1 cm^3 solution of leaf pigments had to be diluted to show the same intensity of colour as the standard solution.

The analyses were made on different samples of each species in three groups, and three samples of each sex of every species were studied in each group. The material used in the three different groups of the tetraploid and hexaploid species originated from three different localities in Scandinavia, but all the material of the diploid species originated from the Portuguese plants of karyotype P_1.

TABLE 19. *Content of chlorophyll in R. sg. Acetosella.*

	2x		4x		6x	
	♀	♂	♀	♂	♀	♂
Group I:	2,30	2,40	5,50	7,00	5,50	5,50
» II:	2,40	2,60	5,60	7,00	5,00	5,10
» III:	2,80	3,00	6,50	5,60	4,90	5,90
Mean:	2,50	2,67	5,87	6,53	5,13	5,50

A very significant difference is noted between the diploid and tetraploid species, positively correlated with the increase in chromosome number. The difference between the tetraploid and hexaploid species, on the other hand, is somewhat negative.

The method used by the present writer is, however, much more inexact than the methods used by GYÖRFFY (1941 b) and PIRSCHLE (1942 a), who found a distinct decrease in the content of chlorophyll per weight unit in a number of experimentally produced autotetraploids and autotriploids. The differences met with in the present material are, however, significant. They and the other results obtained may elucidate the desirability of closer investigations into the physiological conditions of *both* experimental and natural auto- and allopolyploids *of one and the same group of species*, instead of studies of only experimentally produced autopolyploids of one or two races of the species.

G. PHOTOPERIODISM.

Most of the induced autotetraploids show some modifications in the time of flowering, if compared with their diploid sister plants (cf.

STRAUB, 1941). In some of the cases it is not impossible that this difference is merely due to changes in the reactivity to the photoperiod than in the slower growth of the organs. According to ERNST (1941), such a change seems to occur in tetraploid *Antirrhinum*, which shows a long-day reactivity, although its diploid sister plants were short-day plants. This observation, however, has not yet been definitely confirmed.

My material of *R.* sg. *Acetosella* indicates differences in photoperiodical reactivity between at least the diploid species and the polyploid ones. In S.W. Europe it is found to flower in April—June, but in my cultures at Svalöf the diploid species flowers *only* in August—October. The tetraploid and hexaploid species flower from about the middle of May to the beginning of July. It is assumed that these differences are due to the diploid species' being a short-day plant, but the tetraploid and hexaploid species may be long-day plants or show a neutral reactivity to the photoperiod. This assumption is strongly supported by the geographical distribution of the species (cf. Figs. 1—4). The diploid species is found almost exclusively between 55° N. lat. and 55° S. lat., but the tetraploid and hexaploid species are distributed from about 40—50° N. lat. and up to the arctic regions. The octoploid species is only met with in the arctic regions. However, closer studies are needed of strictly autopolyploid forms of one and the same race of *R. angiocarpus* in order to show whether the differences, which are assumed to be due to the increase in the number of chromosomes, are really to be explained in this way.

4. SUMMING UP.

The analysis of the physiological conditions in *R.* sg. *Acetosella*, the only natural polyploid series hitherto studied in this way, shows that the polyploidy affects all the examined characters, except the water content. An increase in chromosome number gives rise not only to an increase in osmotic pressure and p_H of sap, but also to a decrease in the content of ash and oxalic acid, as well as ascorbic acid. The content of chlorophyll is found to increase from 2x to 4x and to decrease from 4x to 6x. The rate of growth of the polyploids is slower than that of the diploid species, and it is found to be slowest in the octoploid species. Differences in photoperiodism, indicating that the polyploidy may change a short-day reactivity to the photoperiod into a neutral or long-day one, seem to arise between the species.

As mentioned above, the distribution of the four species of the subgenus shows a clear correlation between a northern distribution and

polyploidy. If the assumption made regarding the photoperiod and distribution of the diploid species as a whole is correct, a polyploid that also shows a neutral reactivity to the photoperiod will be able to widen the distribution area northwards above 55° N. lat. The present writer assumes that both the tetraploid and the hexaploid species show a neutral to long-day photoperiodical reactivity, but a higher degree of polyploidy — octoploidy — may produce a distinct long-day reactivity, such as must be found in the arctic species of the group. Moreover, the distribution of the different species may be greatly affected by the increase in winter-hardiness met with in the subgenus. The diploid species showed a low degree of winter-hardiness, making it unable to overwinter in the temperate climate of Scania. The tetraploid species showed a higher degree of winter-hardiness, and the hexaploid species was able to survive even the coldest winter experienced in Scandinavia for a long time. These observations are directly correlated with the increase in osmotic pressure met with in this polyploid series, and these two factors may also have greatly affected the distribution of the polyploid species.

The results obtained by the present writer show some differences from the results obtained by previous workers studying experimentally produced autopolyploids. The same general tendency to an increase in some conditions with corresponding decrease in others, as might be expected from the close conformity between the morphological characteristics of different polyploids in nature and experiment, does not apparently exist in the physiological conditions. It is supposed that the different characters show differences in physiological reactivity to polyploidy due to genetical differences between races of the same species. Although the osmotic pressure and other such characters greatly affecting the hardiness, etc. of the plants, will primarily show only an increase in variability in experimentally produced polyploids (cf. GYÖRFFY, 1941 a, b), crosses between such types combined with subsequent selection may result in products which in these important characters considerably surpass their diploid ancestors. The natural polyploid species of *R.* sg. *Acetosella* have been exposed to such a natural selection for at least a million generations.

XII. COMPENDIOUS DISCUSSION.

As mentioned in the introduction to this paper, the three subgenera of *Rumex* are morphologically and cytologically very well distinguished,

according to previous studies of their taxonomy and cytology. The results of the present investigation afford a clearer idea of the biological differences met with between the three groups, as well as of the variation within the sg. *Acetosella*.

According to the studies of the present writer (LÖVE, 1940 b and unpubl.) there are great differences in the size of the chromosomes between the sg. *Acetosa* on the one hand and the two sg. *Acetosella* and *Lapathum* on the other. However, the diploid species of *Acetosa* and *Acetosella* have been found to include differences in karyotypes, but in the diploid species of *Lapathum* no clear evidence for such differences has as yet been found. The barriers of incompatibility noted between the three subgenera are absolute, as no cross products have ever been obtained in nature nor in experiments. The groups *Acetosella* and *Lapathum* are also found to differ from the group *Acetosa* in the frequency of polyploidy, which is almost unknown in *Acetosa* but very common in both the other subgenera.

In connection with the question of differences in karyotypes within the genus *Rumex*, the author wishes to emphasize that he uses the word »karyotype» in the same sense as LEWITSKY (1931 b), KIHARA and YAMAMOTO (1931), YAMAMOTO (1933, 1938), ONO (1935), and others (cf. SHARP, 1934), i. e. as *an indication of the morphological type and form of the chromosomes* of an individual or groups of individuals in the same species or genus, but not as a collective name for the number, size, form, etc. of the chromosomes, as found by BRUUN (1932), nor as a term for differences in chromosome number between closely related species, as found, e. g., by WESTERGAARD (1938 b) and MATSUURA (1935). The karyotypical differences are frequently met with in races with different distribution, as, for instance, in the diploid species of *R.* sg. *Acetosa* section *Euacetosae* in Japan (cf. YAMAMOTO, 1938) and Scandinavia (LÖVE, unpubl.), and in *Vicia* (SWESCHNIKOWA, 1929), and they may differ in segmental interchanges and other chromosomal aberrations as well as in a number of genes.

The differences in karyotypes met with in *Acetosa* and *Acetosella* reveal another interesting feature of the species formation in both these groups. In *Acetosa* the smaller differences in karyotypes are met with in types taxonomically named as varieties or subspecies, but the wider differences in karyotypes are found between the different species of the group (LÖVE, unpubl.). In this subgenus the evolution of species seems to be a gradual process based mainly on rearrangements within the diploid chromosome set. Although the age of the *Acetosella* group is

about the same as that of *Acetosa*, only one diploid species is found in the subgenus, and it is assumed that the karyotype differences in this diploid species possess only a secondary value for the formation of species in this subgenus. The speciation of the two subgenera *Acetosella* and *Lapathum* seems to be based mainly on the polyploidy, but a polyploidy of two different kinds, as will be mentioned below.

The question of which kind of polyploidy occurs in these two groups of *Rumex* is a delicate problem for a long discussion on the differences between auto- and allopolyploidy. The point will be discussed only briefly in this paper, but references are given to the reviews of this problem by DARLINGTON (1937), FAGERLIND (1937), and MÜNTZING (1936). It must, however, be emphasized that in the opinion of the present writer there are two main possibilities in defining the two categories of polyploidy: a definition on a purely cytological basis, or a definition on a wider, both cytological and genetical, basis. A definition of the first-mentioned kind, allowing for one class of autopolyploids in their strictest sense and one class of allopolyploids in their widest sense, is given by MÜNTZING (1936): »The genomes of autopolyploids are structurally identical but may be genetically different . . . Allopolyploidy, on the contrary, involves structural differences between some of the genomes». If this definition were applied, practically all natural polyploids could be classed as allopolyploids, and even hybrids between two experimentally produced autopolyploids of different karyotypes of the same species would have to be classed as allopolyploids. A definition of the second kind, including both natural and experimental polyploids in both the groups, means that *autopolyploids are polyploids originally obtained from a reduplication of the same, cytogenetically mainly homologous, set of chromosomes* (cf. KIHARA and ONO, 1926). It is not impossible that the plant originally reduplicated has been an intraspecific cross, which may or may not have been partially sterile, as some differences may be found in the chromosome complement of different races of one and the same species. *Allopolyploids represent polyploids containing the doubled genome of a hybrid between two well-defined species with cytogenetically somewhat different sets of chromosomes.* The undoubled hybrid will mostly show a high degree of sterility or decreased vitality. As it may be assumed that natural polyploids almost invariably originate from more than one reduplication of more than one race or hybrid of its ancestors, the present writer prefers the last-mentioned definitions as applied to natural polyploids.

In order to control whether the plants investigated belong to the

allo- or autopolyploid category, a number of different methods may be used. Cytologically, the allopolyploids will almost exclusively show autosyndesis, and for the most part they give no multivalents at metaphase-I. The autopolyploids, however, show both allo- and autosyndesis, and multivalents are met with in a fairly high frequency. The fertility is often found to be more disturbed in auto- than in allopolyploids, but the boundary line fluctuates a good deal, as the fertility may also be affected by the size of the chromosomes (KOSTOFF (1940), or by the chiasma frequency (UPCOTT, 1939), or again by genotypical control of bivalent formation (MÜNTZING and PRAKKEN, 1940). The size of cell is found to increase more in autopolyploids than in allopolyploids (cf. WETTSTEIN, 1940; KIHARA and ONO, 1926); consequently the different organs show a relatively greater increase in auto- than in allopolyploids.

A very significant difference occurring between natural and experimental autopolyploids is the high degree of sterility in the last-mentioned group (cf. STRAUB, 1940, 1941; MÜNTZING, 1936, 1937, 1939 b; KOSTOFF, 1941). According to MÜNTZING (1936), these differences are due to natural selection, possibly combined with secondary processes of various kinds. This hypothesis is strongly supported by the results obtained by RANDOLPH (1941) in *Zea* and by MÜNTZING (1943) in *Galeopsis*, where it was found that »the fertility of autotetraploids may be increased by recombination following hybridization». It is also supported by the assumption that natural autopolyploids hardly ever originate from a single doubled line; all natural polyploids are presumably of polyphyletical origin.

The differences in kind of polyploidy between *Acetosella* and *Lapathum* may be discussed on the basis of the above-mentioned definitions. Unfortunately, only a few investigations have as yet been made into the sg. *Lapathum*. Most of the polyploid species of this subgenus hitherto studied show no multivalents at meiosis, and the size of the cells (pollen grains) is not found to increase proportionally to an increase in chromosome number. They are, therefore, regarded as extreme allopolyploids (KIHARA and ONO, 1926), but autopolyploids may also be met with in the group (LÖVE, unpubl.). The differences in species within the sg. *Lapathum* seem to be due partly to a gradual differentiation of the chromosomes in the diploid species, but mainly to (allo-)polyploidy. The differentiation of the few diploid species of the group is assumed to be much older than the polyploidy in this subgenus,

a hypothesis which may in some measure explain the high frequency of allopolyploids in the group.

According to the present investigation, however, *the differentiation of species in sg. Acetosella is solely due to autopolyploidy.* This statement is supported mainly by the following observations: The group involves only one diploid species, but three polyploid species, all having different chromosome numbers and differing in only small quantitative characteristics. The type and size of the chromosomes is the same in all the species, and the sex chromosomes and sex mechanism are also identical. The meiotic behaviour of the three species examined shows that in the form of the diploid species studied the highest number of ring-bivalents is three. It is assumed that an autopolyploid of a diploid with small chromosomes and only rod-bivalents (= 0,5 chiasmata per chromosome) will form no multivalents at meiosis, but that an autopolyploid of a diploid with small chromosomes and, e. g., three rod- and three ring-bivalents will never form more than three multivalents at meiosis, presuming that heterozygosity for segmental interchanges does not occur. The observations of the highest number of multivalents at meiosis in the polyploid species of the *Acetosella* group agree with this assumption. The hypothesis on the autopolyploidy of the group is also strongly supported by the observations of the high frequency of pairing (auto- and allosyndesis) in the pentaploid hybrids between the tetraploid and hexaploid species, as well as by the results of the studies of the minor morphological differences between all the species.

It may perhaps be remarked that the observations of the meiotic behaviour of the tetraploid and hexaploid species do not always coincide with the hypothesis of their autopolyploid origin. Practically no two plants of the hexaploid species show the same meiotic behaviour, and most of the plants of the tetraploid species proved to be different from all the other individuals studied. Inversions and lack of homology between one or two pairs of chromosomes are not rare, and heterozygosity for one and even two segmental interchanges is fairly common in both the polyploid species examined. The present writer assumes, however, that these differences do not form a contradiction to the hypothesis of the autopolyploid origin of the species, as they may be explained in either — or both — of the following ways:

Firstly, the polyploid species have not been formed by a single reduplication of a single karyotype of the diploid species, but by a great many reduplications of a number of karyotypes. According to the present investigation, the karyotypes of the diploid species are found

to differ in segmental interchanges, and within the morphologically inseparable karyotypes heterozygosity for inversions and other smaller chromosomal aberrations may be found. The karyotypes of the diploid species may, theoretically, differ in the frequency of ring-bivalents, i. e. in chiasma frequency. A strict autotetraploid form of karyotype P_1 will, for instance, form three quadrivalent autosome-associations as a maximum, but a strict autotetraploid of a karyotype with, e. g., only one ring-bivalent will never form more than one quadrivalent. Hybrids between two such strict autotetraploids may form associations of more than four chromosomes, inversion bridges, only bivalents or some univalents, etc. It is, however, to be regarded as an autopolyploid in the wider sense. Presumably, most of the individuals of the tetraploid species and practically all the plants of the hexaploid are heterozygous for two or more karyotypes.

Secondly, the high age of this polyploid series (cf. below) makes it not inconceivable that a number of chromosomal rearrangements may have occurred within each species since their formation, causing them to tend towards secondary allopolyploidy. This assumption is supported, e. g., by the observations on the pairing property of the *X*-chromosomes in the polyploid species. It is also supported by the observations of »cytomixis» and inversion bridges in the present material, as both these phenomena may affect the arrangements within the chromosomes. Moreover, after hybridization between two karyotypes new forms of chromosomes may occur (cf. SINOTO, 1937; YAMAMOTO, 1938), and after hybridization between polyploid types differing in segmental interchange the frequency of multivalents may be greatly reduced, owing to genetical non-disjunction in the rings or chains.

The author suggests that both the above-mentioned possibilities cause the meiotic variability of the tetraploid and hexaploid species. The former is, however, presumably the most frequent one, although the significance of the latter is not to be underestimated. The polyploid species are without doubt on the way to secondary allopolyploidy. They are still, however, to be regarded as autopolyploids, as is shown by the complete pairing in their pentaploid hybrids.

The diploid species of sg. *Acetosella* shows by far the largest distribution area. This is assumed to be caused partially by the high age of the species and partially by the fact that this weed is the only species of the group found in the seaports in S.W. Europe with a very lively transoceanic traffic throughout many centuries. However, the polyploid species are also found to have a large distribution area, but they

are mainly met with in latitudes uninhabitable by the diploid species. In particular, the distribution of the octoploid type, which is frequent in arctic regions never reached by the last glaciation (cf. HULTÉN, 1937), shows that the polyploidy within this group must be of very ancient date. Before the last Ice Age began about one million years ago the polyploids of sg. *Acetosella* must have been distributed over very large areas, without doubt much larger than those existing at present.

Although the distribution of the polyploid species is certainly an indication of a high age, it is not impossible that the polyploid species may be evolving even at the present time. The author holds the view that they have been formed again and again in the course of the ages, and the formation of new polyploids in nature will never cease. This assumption is to some extent supported by the observations of unreduced gones in the species studied.

As mentioned in the introduction, the taxonomists studying the *Acetosella* group during the last century were not able to survey all the variations met with in the group. According to the present investigation, however, four biologically isolated groups of variation are met with in the subgenus. These groups are regarded as four different species.

According to the works of a number of cytogeneticists, such as, for instance, DARLINGTON (1937), FAGERLIND (1937), KOSTOFF (1941), and MÜNTZING (1936), the subgenus *Acetosella* should be regarded as a very good example of »intraspecific polyploidy». The term is defined by FAGERLIND (l. c.) as follows: »Intraspecifische Polyploidie liegt vor, wenn die n-Chromosomengarnitur zusammensetzenden Genome von derselben Art herstammen». This conception merely serves to add to the already existing confusion in regard to the problem of the taxonomical significance of chromosome numbers. If this definition were generally accepted by taxonomists, not only would all collective species with more than one chromosome number be regarded as one well-defined species, but also a number of Linnaean species would have to be united into one species, as they have been found to form an autopolyploid series. According to other views, however, the function of cytogenetics is to help us to detect and understand biological isolations between and within different species, and that of taxonomy is to help us to recognize the biologically isolated groups.

The present writer agrees with the last-mentioned opinion. When differences in chromosome number are detected within an old species, the different types must be closely studied morphologically. If they are found to differ in some taxonomically useful characteristics, they have

to be described as different species, according to the principle enunciated by NANNFELDT (1938): »As soon as chromosomal races ('polyplotypes') are morphologically distinct and thus recognizable to the taxonomist, they had better be regarded as species even if the morphological characters are small» (cf. also LÖVE and LÖVE, 1942 b). On the basis of these principles the four biologically isolated groups of sg. *Acetosella* are regarded as taxonomically good species, differing in small but distinct quantitative characteristics. The present writer goes so far as to doubt whether any polyploid series exists without some taxonomically useful differences.

The evolution of the two dioecious groups of *Rumex* may also be discussed from the standpoint of the differences in sex mechanisms met with between them. The two groups coincide with the fundamental theory of sex determination, as one of the sexes is found to be heterogametic. They disagree, however, in the fundamental point of localization of the sex-determining genes. In sg. *Acetosa* no sex-determining genes are found in the *Y*-chromosomes, and the male-determining elements (*M*) are only found in the autosomes. The female-determining elements (*F*) are found in the autosomes and in the *X*-chromosomes. The total sum of *F*-determinants in a diploid is higher than *M*, if both *X*'s are found in the same individual, but if one *X* is absent the total sum of *M* is higher. Therefore, a $2A + 2X$ individual is a female when a $2A + X$ individual is a male. The differentiation of the sexes in this type of sex determination from the more primitive state to the dioecious one may be regarded as the final result of a successive mutation process along practically the same lines as suggested by CORRENS (1928), though somewhat modified. The mutation process mainly involves the *F*-determinants or the strength of the *F*-determinants in this type. Then, the hermaphroditic species which has developed into a dioecious one of the *R. Acetosa* type must primarily have changed into a gynodioecious race with only female and hermaphroditic individuals (cf. the terminology of YAMPOLSKY and YAMPOLSKY, 1924; MONIUSZKO, 1937), which again will successively change into the polygamodioecious state with females, males and hermaphrodites. Gynodioecious and polygamodioecious species have so far been found within the section *Hastati* of the sg. *Acetosa*. As has been shown by CORRENS (1928) and ONO (1940 b), the hermaphroditic (and male) plants of this type are always the heterogametic sex. They may or may not have visible sex chromosomes, but perhaps these will not develop before the hermaphroditic plants are fully eliminated by selection and subsequent changes in the

sex genes. The evolution of visible sex chromosomes is without doubt a secondary phenomenon, and will never be found in a number of plants and animals.

In *R.* sg. *Acetosella* the strongest *M*-elements are found in the *Y*-chromosome, but, theoretically, *M*-elements may also occur in the autosomes. The *F*-elements are assumed to exist in the *X* as well as in the autosomes. The *M*-elements in the *Y*-chromosome have such a strong epistatic power that they can entirely suppress the total sum of the *F* in the hexaploid and the octoploid species. The evolution of this type of sex determination is assumed to have passed from the hermaphroditic state via the polygamodioecious and the androdioecious (males and hermaphrodites) ones to the dioecism, as the successive mutation process in this type must have gone on primarily in the *M*-determinants. Theoretically, if the above hypothesis is right, the hermaphrodite individuals of androdioecious species must be homogametic, and the males must be heterogametic. The main difference between these two hypothetical types of evolution of the sex mechanisms is that *the mutation process in the Acetosa type must have gone on mainly in the female direction*, forming an excess of *F*-genes in the *X*-chromosomes, *but in the Acetosella type they have mainly turned in the male direction*, forming the epistatic *M*-genes in the *Y*.

According to this hypothesis, the two types of sex determination met with in the dioecious groups of the genus *Rumex* are regarded as being due to two opposite directions of evolution of the localization and development of the sex genes. As these two evolutional processes must have been different from the beginning, they are to be regarded as going on parallelly to one another, but not as different stages of the same process.

The two types of localization of sex-determining genes mentioned above are the only ones studied as yet, but they are certainly not the only types found in the plant and animal kingdoms. The same type as that found in *R. Acetosa* is also met with in *Drosophila*, while the *Melandrium—Acetosella—*(*Achnida*) type may be expected in the genera *Vallisneria* (cf. WESTERGAARD, 1940) and *Welwitschia* (cf. FERNANDES, 1936), and perhaps also in *Populus* (cf. BLACKBURN, 1929; JOHNSSON, 1940), *Salix* (cf. HARRISON, 1926; SINOTO, 1929), and *Urtica* sect. *dioeca* (cf. LÖVE and LÖVE, 1942 a). Moreover it is presumably the most frequent sex mechanism in the higher animals with a relatively high chromosome number (cf., e. g., Fig. 95 by STURTEVANT and BEADLE, 1940). Some other kind of sex mechanism must, however, be found in

Hymenoptera and certain Homoptera with diploid females and haploid males, as haploids with $A + X$ must, theoretically, be females if the sex determination was in accord with the *Drosophila*- or *Melandrium*-scheme. The mosses also are certainly different from these two types, and if the explanation given by HAGERUP (1927) on the origin of *Empetrum hermaphroditum* is right, this species too belongs to a type of localization of sex genes other than the above-mentioned types. It is not impossible, however (cf. WESTERGAARD, 1940) that the dioecious, diploid *E. nigrum* and the tetraploid *E. hermaphroditum* have developed as two different lines from the same diploid, hermaphroditic species. This putative common ancestor may or may not be extinct (cf. BLACKBURN, 1938).

On the basis of all the above-mentioned results and hypotheses the present writer regards it as definitely demonstrated that the sg. Acetosella is no more closely related to the sg. Acetosa than to the sg. Lapathum. He further assumes that these three subgenera may have developed from a common ancestor as three different but parallel lines of evolution. The three polyploid species of sg. Acetosella have developed from the diploid one by autopolyploidy. They are, according to their present distribution, very old species, differing in distinct quantitative characteristics.

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SUMMARY.

1) The subgenus *Acetosella* of the genus *Rumex* has been found to form an autopolyploid series with the basic number $x = 7$. The four species of the group are: *R. angiocarpus* ($2n = 14$), *R. tenuifolius* ($2n = 28$), *R. Acetosella* s.str. ($2n = 42$), and *R. graminifolius* ($2n = 56$). The diploid species show a southern distribution, but the octoploid species is only met with in arctic regions. There is evidence that karyotypical differences exist in the diploid species.

2) The meiotic behaviour of the diploid species is mostly normal, but irregularities caused by heterozygosity for segmental interchange and inversion as well as partial asynapsis and some minor disturbances have been observed. The type with segmental interchange is regarded as a hybrid between two karyotypes, but the other irregularities are found in morphologically symmetrical karyotypes.

3) In *R. tenuifolius* univalents are found at meiosis in almost all the plants studied. Inversion bridges are found in 52 % of the material studied, and heterozygosity for segmental interchange is found in about 33 % of the material. Three of these plants are heterozygous for two segmental interchanges.

4) In *R. Acetosella* s.str. irregularities are met with in almost all the individuals. Inversion bridges are found in 14 out of 15 plants studied, and two plants are heterozygous for two inverted segments; 33 % of the individuals are heterozygous for segmental interchange.

5) Two pentaploid hybrids between *R. Acetosella* s.str. and *R. tenuifolius* show a high frequency of pairing due to auto- and allosyndesis, but in almost all the cells there is pairing of chromosomes that might be expected to be univalents. The possible causes of this phenomenon are discussed.

6) In all the species the pollen fertility is found to be very good. Moreover, plants with numerical non-disjunction show almost complete fertility of the pollen grains. In the diploid plant with segmental interchange numerical non-disjunction is found in 51 % of the cells, but the

pollen is 99,0 % good. A plant of *R. Acetosella* s.str. with marked irregularities in the distribution of chromosomes at anaphase (n = 18—23) shows 93 % good pollen, which was found to germinate up to almost 100 % of the filled grains. This plant was used as a father plant in a cross experiment. Only three of the six expected chromosome numbers in the progeny were realized. The pollen fertility of the pentaploids was almost as good as that of the parent species, but no seeds could be obtained from the female plants. The chromosomal sterility of the subgenus seems to be of the diplontic type, but not of the haplontic type found in almost all plants previously studied.

7) Cross experiments between the different species yield no results, except when the hexaploid is pollinated with pollen from the tetraploid.

8) No evidence of the occurrence of agamospermy is found in my material. According to previous studies, however, the possibility is not entirely excluded in some forms.

9) Sex-linked heterochromosomes are found in the males of all the species. They are of the *XY* type, and the *Y* is the larger one, as has previously been found in a few plants.

10) The localization of sex determinants is of the same type as that found in *Melandrium* with strong male elements in the *Y*. This type of localization is not met with in *R. Acetosa*, which belongs to the *Drosophila* type.

11) The polyploid species have one *Y*-chromosome but three, five or seven *X*-chromosomes in the male plants. They are, however, dioecious, owing to the very strong epistatic male elements in the *Y*-chromosome.

12) The mode of pairing of the sex-chromosomes in the polyploid species shows that the extra *X*-chromosomes gradually differentiate into normal autosome pairs.

13) Intersexes with 2n = 42 chromosomes and no detectable deviations in meiotic behaviour from the normal male plants are met with. They are assumed to be the results of the accumulation of female-determining genes in the autosomes.

14) The sex ratio of *R.* sg. *Acetosella* shows practically no deviations from the expected 1 : 1 ratio.

15) The four species are found to differ in a number of minor morphological characteristics, which seem to be of a purely quantitative kind.

16) The size of the nucleus, stomata, pollen, female flowers, anthers, and fruits is found to increase proportionally to an increase in the chro-

mosome number, except the stomata in the octoploid species, which may decrease somewhat.

17) The chloroplasts in the 2x, 4x and 6x show no statistically demonstrable differences in size, possibly owing to their genetical independence.

18) The rate of growth of this polyploid series is found to become slower with an increase in the chromosome number.

19) The osmotic pressure and p_H of sap are found to increase proportionally to the increase in the chromosome number, but the content of ash, oxalic acid and ascorbic acid decreases with an increase in the chromosome number. No relations were detected between content of water and chromosome number.

20) The content of chlorophyll is found to increase from 2x to 4x but to decrease from 4x to 6x.

21) There is evidence which indicates a relation between chromosome number and photoperiodical reactivity. The diploids are short-day plants, the polyploids are neutral or long-day plants.

22) The winter-hardiness is found to increase as the polyploidy increases. This is found to be correlated with the distribution of the species. The distribution may be affected by the differences in osmotic pressure and photoperiodical reactivity.

23) Differences between the sexes are observed in the content of ash, ascorbic acid, oxalic acid, and chlorophyll.

24) The evolution of the three subgenera of *Rumex* and the four species of sg. *Acetosella* is discussed on the basis of the results obtained by the present and previous investigators. The three subgenera are regarded as three parallel but different lines of evolution. The polyploid species of sg. *Acetosella* are very old species, which have originated from the diploid species by autopolyploidy. They are, however, on the way to secondary allopolyploidy.

25) The problem of »intraspecific polyploidy» is discussed at some length. The writer considers that all »intraspecific polyploids» may be distinguished as different species. He agrees with the opinion set forth by NANNFELDT (1938).

26) Some general remarks on the evolution of sex mechanisms are made on the basis of the present results. The author regards the two types of sex mechanism met with in *Rumex* as being due to two opposite directions of evolution.

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CHROMOSOME BEHAVIOUR IN A TRIPLOID RYE PLANT

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THE present investigation was made in the summer of 1936. The material, a twin pair of rye plants consisting of one diploid ($2n = 14$) and one triploid ($2n = 21$), was obtained from Professor A. MÜNTZING, to whom I am very much indebted. I am also indebted to Dr. K. MATHER, with whom I have discussed the problems of negative correlation. MÜNTZING (1937) has briefly reported some interesting circumstances concerning these twin seedlings. Here, above all, the surprisingly good pollen-fertility of the triploid twin plant should be mentioned.

Morphologically the triploid and diploid individuals were rather difficult to distinguish from each other. Exact measurements were not made, but possibly the triploid plant was slightly more vigorous.

Anthers of the triploid plant were fixed in alcohol-acetic acid (3 : 1) and the sections stained with gentian violet.

Chromosome behaviour. — The first metaphase of meiosis was analysed in side views of twenty nuclei. The following pairing types were found:

Configuration	No. of cells
$1_I + 1_{II} + 6_{III}$	3
$2_I + 2_{II} + 5_{III}$	5
$3_I + 3_{II} + 4_{III}$	3
$4_I + 4_{II} + 3_{III}$	4
$5_I + 5_{II} + 2_{III}$	4
$7_I + 7_{II}$	1
	Total 20 cells

The number of trivalents per cell varied from 0 to 6, the mean number being $3{,}75 \pm 0{,}36$.

Two complete nuclei of the triploid rye plant are illustrated in Figs. 1 and 2, where the different types of configurations are marked by letters. In the twenty nuclei the total frequencies of these configurations were as follows:

Type of configuration	No. of xata per configuration	Corresponding letter in Figs. 1 and 2	Total number of configurations in 20 nuclei
Univalents	0	*u*	65
Rod bivalent	1	*a*	6
» »	2	*b*	1
Ring »	2	*c*	38
» »	3	*d*	20
Chain trivalent	2	*e*	29
» »	3	*f*	10
Frying pan trivalent	3	*g*	31
» » »	4	*h*	5

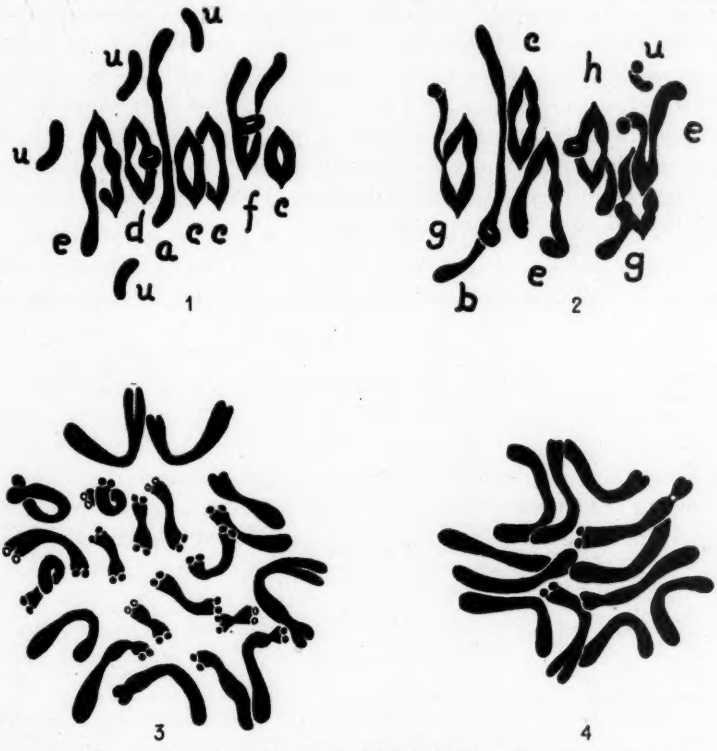
Y-trivalents have not been observed. In view of the unexpectedly high frequency of frying pans the absence of Y-trivalents is surprising. TAKAGI (1935) has observed in trisomic rye the occurrence of Y-trivalents.

From the frequencies given above of various configurations at first metaphase the mean number of half-chiasmata per chromosome has been determined as 1,64. In the case of frying-pan trivalents there is more than one possible distribution of half-chiasmata among the chromosomes (DARLINGTON and MATHER, 1932), and accordingly one maximum and one minimum variance might be calculated (UPCOTT, 1935). From the mean 1,64 these variances have been determined as 1,023 and 0,827 respectively. In the last column of Table 3 the chiasma frequencies have been expressed as the number of chiasmata per nucleus. This last method of expression has given a mean of 17,25 with a variance of 3,355. The terminalisation of the triploid rye plant expressed as terminalisation coefficient was 0,70.

At first metaphase the mean number of univalents per cell was $3{,}25 \pm 0{,}369$. Lagging univalents were frequently seen at first anaphase. These univalents may or may not divide, the ratio of division and non-division counted on 42 cases being 3 : 4. Lagging univalents were also observed during second anaphase, these presumably being divided univalents from the first division. Here the exact frequency has not been determined, but in a lot of pollen mother cells there were two such lagging univalents between each of the two anaphase groups. A number of the univalents at second anaphase never reach the poles and are later seen as micro-nuclei in the tetrads of the young pollen-grains.

The frequency of micro-nuclei in the young pollen-grains is given below.

Micro-nuclei per pollen-grain ..	0	1	2	3	
No. of pollen-grains	52	69	26	3	Total 150 grains.



Figs. 1—2. Two complete nuclei of the triploid plant at first metaphase, with the following types of configurations: univalents (*u*), rod bivalents with 1 xa (*a*) and 2 xata (*b*), ring bivalents with 2 xata (*c*) and 3 xata (*d*), chain trivalents with 2 xata (*e*) and 3 xata (*f*), frying pan trivalents with 3 xata (*g*) and 4 xata (*h*). — Fig. 3. Somatic plate of triploid rye with twenty-one chromosomes. — Fig. 4. Somatic plate of the first mitotic division in the pollen-grains of the triploid rye plant with twelve chromosomes. — × 3050.

The mean number of micro-nuclei per pollen-grain was $0{,}87 \pm 0{,}063$, which corresponds to a minimum elimination of 24,9 per cent of the seven odd chromosomes of the triploid. As the tetrads sometimes also contain microcytes, which had not been counted among the young

pollen-grains, the true elimination was probably somewhat higher. The elimination has also been determined by counting the number of chromosomes at the first mitotic division of fifty pollen-grains. The mean number of chromosomes per pollen-grain was $9{,}46 \pm 0{,}229$, which corresponds to an elimination of 29,7 per cent of the seven odd chromosomes of the triploid. Accepting this percentage of elimination, the expected random distribution of chromosome numbers of the pollen-grains has been calculated by the formula $50(p + q)^7$, where q is equal to $(9{,}46 - 7{,}00) : 7 = 0{,}35143$. In Table 1 this expected distribution by the chi-square method has been compared with the distribution actually observed. There is a significant deviation, chiefly owing to the deficiency of pollen-grains with ten chromosomes. This deficiency, however, might be due to accidental circumstances.

TABLE 1. *The distribution of chromosome numbers in fifty pollen-grains of a triploid rye plant.*

No. of chromosomes	Frequencies observed	Frequencies expected	Chi-squares	
7	4	2,41	2,5600	
8	13	9,15		
9	12	14,89	0,5609	
10	6	13,44	4,1186	
11	9	7,28		
12	4	2,37	2,3652	
13	2	0,43		
14	—	0,03		
Total	50	50,00	9,6047	D.F. = 2. P = 0,05 — 0,02

The somatic mitosis of the pollen-grains was studied in slides of smeared anthers. In contradistinction to the somatic metaphases of the root tip slides it often looked as if homologous chromosomes were somatically paired (see Figs. 3 and 4), but more careful investigations are necessary to corroborate this observation. Data relating to the pollen size are given in Table 2, which shows the diameter of the pollen-grains of the diploid and triploid twin plant. Every measurement has reference to 200 pollen-grains.

The mean diameter of the pollen-grains of the triploid twin plant

TABLE 2. *Diameters of 200 pollen-grains of the diploid and triploid twin plant (1 unit = 1,8 μ).*

	Diameters of the pollen-grains in units.														
2n	18	19	20	21	22	23	24	25	26	27	28	29	30	31	M ± m
14		1	1	1	4	9	34	61	51	32	5	1			$25{,}28 \pm 0{,}10$
21	1			1		7	24	48	42	36	23	14	3	1	$26{,}06 \pm 0{,}13$

was significantly larger than that of the diploid ($t = 4{,}848$; $P < 0{,}001$). The size difference, however, is not very accentuated.

Mature pollen was examined in a mixture of glycerine and aceto-carmine. The grains which were full of stained material were counted as »good» and those which were irregular in outline, imperfectly filled or empty, were counted as »poor». In the triploid plant 306 grains were examined, 83 per cent of which were »good». The corresponding determination on 210 pollen-grains of the diploid plant gave 96 per cent good grains.

The triploid plant, however, was completely self-sterile, and it did not function as male parent in crosses with diploids. As mother plant it gave a few diploid descendants when crossed with diploid rye.

Negative correlation of chiasma frequencies. — Negative correlation of chiasma frequencies has been shown in diploid rye by MATHER and LAMM (1935) and LAMM (1936). The triploid plant of the present investigation has also been analysed from this point of view. In triploids, according to MATHER (1936), the potential trivalent should be the basis of the analysis (see Table 3), since if the determinations of half-chiasmata per chromosome were used, there would be a confounding of the intra- with the inter-trivalent variance.

TABLE 3. *Chiasmata per potential trivalent in the pollen mother cells of a triploid rye plant.*

No. of p.m.c's	Xa per potential trivalent				Total No. of xata per p.m.c.
	1 xa	2 xata	3 xata	4 xata	
1	2	4	1		13
1 [1]	1	4	2		15
4		5	2		16
1	1	2	4		17
1	1	3	2	1	17
3		4	3		17

[1] Compare Fig. 1.

No. of p.m.c's	Xa per potential trivalent				Total No. of xata per p.m.c.
	1 xa	2 xata	3 xata	4 xata	
1	1	2	3	1	18
1 [1]		4	2	1	18
5		3	4		18
1		2	4	1	20
1			6	1	22
Total 20	6	68	61	5	345

Analysis of variance of the figures of Table 3 has given an intra-cell variance of 0,3976 and an inter-cell variance of 0,4793, the corresponding degrees of freedom being 120 and 19. The ratio of the large variance to the smaller variance is not significant if tested by the readings from a 5 per cent table of z (FISHER and YATES, 1938, p. 31).

According to MATHER (1936), a negative correlation must indicate some influence of one bivalent on chiasma formation in other bivalents of the same nucleus irrespective of outside influences. This phenomenon has been referred to as »competition». The hypothesis of competition implies that there is an upper limit to the number of chiasmata which can form in any nucleus, the limits varying slightly from nucleus to nucleus. The bivalents or other configurations must compete for these chiasmata.

In comparison with the diploid population rye plants, for which data of chiasma frequencies are given by LAMM (1936), the triploid rye plant has a high number of chiasmata per nucleus. As to the population plants Pa—Pf (compare LAMM, 1936, Table 3 b), the difference is significant, but the plants Pg and Pp (LAMM, 1936, Table 3 b), which were grown in a green-house, have slightly higher mean numbers of chiasmata per nucleus than the triploid twin plant. In *Triticum*, MATHER (1935) has shown that the frequencies of chiasma formation vary from day to day. As the triploid was not fixed in the same year as the diploid population plants grown under the same conditions, it is impossible to tell with certainty whether it has a comparatively high number of chiasmata per nucleus or not. The former assumption seems, however, most likely, but there is no competition. The upper limits of the chiasmata in the nucleus must, if the hypothesis of competition is true, have been raised in the triploid. Investigations on *Tulipa* (UPCOTT, 1939) indicate, however, that the hypothesis of competition might be wrong.

[1] Compare Fig. 2.

Discussion. — The general chromosome behaviour of the triploid plant has not shown any remarkable features save the occurrence of a high frequency of frying pan trivalents and the absence of Y-trivalents. In comparison with diploids (see LAMM, 1936, Table 2) the mean number of half-chiasmata per chromosome was comparatively low. (In diploids the mean number of chiasmata per bivalent and the mean number of half-chiasmata per chromosome agree.) Direct values, however, are not comparable, since with an infinite number of pairing units the number of half-chiasmata in the triploid should be $^2/_3$ of the corresponding diploid number (STONE and MATHER, 1932). From this point of view the chiasma frequency of the triploid as compared with the earlier investigated diploid plants appears, if anything, to be high. An analogous comparison of the terminalisation coefficients shows a comparatively high coefficient for the triploid.

The characters of the triploid pollen-grains are rather interesting. The slight difference in diameter between triploid and diploid pollen indicates a low morphological effect of the odd chromosomes. Most surprising, however, is the high percentage of morphologically »good» grains. This point has been discussed by MÜNTZING and PRAKKEN (1941). On the other hand, this so-called good pollen was not functional when the triploid was used as male parent in crosses.

Finally, the competition should be discussed. In trisomic maize MATHER (1939) has shown that the addition of an extra chromosome raises the upper limit of the number of possible chiasmata. The same phenomenon has now been shown in triploid rye, although here the comparisons between the triploid twin plant and diploid population plants are somewhat doubtful.

SUMMARY.

Meiosis in a triploid twin plant of rye is described.

From nought to six trivalents occur. The trivalents are either of the chain or the frying pan type, the frequency of the latter type being unexpectedly high.

Some, but not all, of the univalents divide at first anaphase and lag at second anaphase. There is a considerable elimination of the odd chromosomes of the triploid. The extent of this elimination has been determined by counting the numbers of micro-nuclei in the young pollen-grains and the numbers of chromosomes in the somatic pollen-mitosis.

The frequency of chiasma formation is determined and expressed both in terms of half-chiasmata per chromosome and number of chiasmata per nucleus. The terminalisation coefficient of the triploid was high.

Mature pollen of the diploid and triploid twin plants was examined in a mixture of glycerine and acetocarmine. The number of »good» grains in the triploid was surprisingly high. As male parent, however, the triploid plant was completely sterile. Data relating to the pollen size of the diploid and triploid twin plants are given.

The addition of an extra set of chromosomes in the triploid is shown to lead to less competition for chiasmata between the chromosomes. The upper limits of the chiasmata in the nucleus have probably been raised in the triploid.

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THE TERMINOLOGY OF THE APOMICTIC PHENOMENA

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IN a paper of 1940, FAGERLIND (1940 a) gave a survey of terms and definitions used up to that time in the embryological and cytological researches on apomixis. In that and in a subsequent publication (1940 b) he criticized rather harshly certain opinions and results put forward by GUSTAFSSON in 1935 and 1939. At the same time he ventured to draw up a new terminological system, which consisted in part of misused terms and for that reason cannot be accepted; in part — when it was not incorrect — it was unsuitable from a general point of view. The misuse of terms depends on the author's obvious ignorance of the works of HARTMANN and others in the field of sexuality and reproduction.

In his review of apomictic problems, STEBBINS (1941, p. 507) accepted FAGERLIND's system in full. »In the opinion of the present reviewer, FAGERLIND's system is satisfactory in every way and should be generally adopted in order to secure uniformity in the description of these phenomena.» In spite of his remarkable piece of work in *Antennaria* and *Crepis*, STEBBINS shows a similar ignorance of previous literature (the publications of EDMAN, 1931; HARTMANN, 1929, 1933; HÄMMERLING, 1940, are apparently unknown to him, although he cites EDMAN's paper). It is in fact surprising how many biologists (even of prominent position) are unfamiliar not only with the inferences drawn by HARTMANN and his colleagues but also with the actual position of sexuality in thallophytes and archegoniates.

The general term *apomixis* is defined by FAGERLIND as »reproduction without fertilization and alternation of nuclear phases». This definition is however too wide, since WINKLER (1908) restricted the expression to plants (and animals) which are primarily sexual and which first after secondary processes have lost their ability of fertilization and alternation of nuclear phases. WINKLER lays special stress upon the point that apomixis is »*den Ersatz der geschlechtlichen Fortpflanzung* durch einen anderen, ungeschlechtlichen nicht mit Kern- und Zellverschmelzung verbundenen Vermehrungsprozess». Organisms

which occupy such a primitive position in the genealogical system that they have not even acquired sexuality are called amictic, and their mode of propagation is called *amixis*. WINKLER (1942) has already objected to the incorrect application of his expression.

Apomixis in higher plants is divided into two groups of phenomena: *agamospermy* and *vegetative multiplication* (TÄCKHOLM, 1922). In the first instance the propagational bodies consist of seeds, in the latter instance they are strictly vegetative and arise outside the ovule and the generative system (bulbs, bulbils, suckers). These terms are correctly applied also by FAGERLIND (and STEBBINS).

Agamospermy consists, according to FAGERLIND (1940 a), of two processes: *agamogony* and *nucellar embryony*. The former is defined as the special case of agamospermy where the reproduction of the sporophyte takes place *via* the formation of an unreduced gametophyte. In nucellar embryony different generations do not alternate, but the new sporophyte is formed directly from the sporophytic tissue of the mother-plant. The term agamogony was, however, previously introduced into the scientific discussion by HARTMANN (1929, 1933). It covers quite another group of phenomena. Therefore HARTMANN's definition has priority. Agamogony, in the correct meaning, was also used by HÄMMERLING in 1940.

The reproduction of an organism, simple or differentiated, takes place, according to HARTMANN, by two means: either by undifferentiated cells (agametes, zoospores, akinetes, gonids), or by special, sexually differentiated cells (gametes). In the latter case the sexual reproduction is combined with an act of fertilization. The first process is called agamogony and occurs among protists and thallophytes, the second is called gamogony and is a universal phenomenon among higher plants and animals. Gamogony may become lost by secondary processes so that for their survival the organisms are referred to single vegetative cells, detached groups of cells (vegetative multiplication) or to the development of unfertilized gametes. Evidently we find here close parallels to the processes given the names amixis, mixis and apomixis by WINKLER. FAGERLIND's term agamogony, used in the aforementioned sense, must be discarded.

According to FAGERLIND, gametophyte formation takes place in higher plants by three part-processes, viz. by *apospory*, *semi-apospory* and *diplospory*. The definitions run as follows: Apospory implies a mode of gametophyte formation in which the first division of the mother-cell is highly mitotized. In semi-apospory the first division is

pseudohomeotypic. In diplospory, finally, it has a pronounced meiotic character (restitution nuclei are formed). Apospory in its turn is either *generative* or *somatic*, dependent on the place of origin of the gametophyte initials.

The term diplospory is thus defined in a manner deviating from what its maker stipulated. The original meaning is quite simply that in the case of diplospory the unreduced gametophyte initials arise in archesporial tissues (EDMAN, 1931). In plants with unicellular archespore (*Taraxacum*, *Hieracium*, *Antennaria*) the diplosporous gametophytes originate from the only and single macrospore (embryo-sac) mother-cell, whether the first division of the EMC is mitotic, pseudohomeotypic or meiotic with the formation of restitution nuclei as a consequence.

Apospory implies, according to EDMAN, that an extra-archesporial (= somatic) cell grows out to a gametophyte initial, which later on will produce embryo-sac, egg-cell and embryo. This definition applies equally well to pteridophytes, where gametophytes and sporophytes grow independent of one another.

FAGERLIND says (1940 a, p. 2): »Ist es erlaubt, auf diese Weise einmal definierte Ausdrücke ihre Bedeutung ändern zu lassen? Ja, unter gewissen Umständen . . . Soweit als möglich muss indessen auf die Priorität stets Rücksicht genommen werden. Sinnänderungen ohne begründeten Anlass sind unstatthaft». The word diplospory was introduced and strictly defined by EDMAN in 1931, correctly used by GUSTAFSSON in 1939, and then abruptly applied to a different process in spore formation by FAGERLIND in 1940. This misapplication shows lack of respect for priority. HÅKANSSON (1943) has already criticized the wrong application of EDMAN's term.

FAGERLIND's terminology is inappropriate in several other respects. Consider, for instance, a hawk-weed belonging to *Archieracium*. (1) The nucleus of the embryo-sac mother-cell may divide early and give rise to normal meiotic divisions with tetrad formation as a consequence. (2) Or the nucleus of the mother-cell may rest for a considerable time in the interphase stage and later on pass through a rapid and strict mitosis. (3) Now and then restitution nuclei seem to arise, at least according to BERGMAN (1941). (4) Similarly pseudohomeotypic divisions are not all too rare (GUSTAFSSON, 1935, 1942). (5) In addition to these modes of gametophyte formation GENTCHEFF (1937) postulated the origin of embryo-sac initials from extra-archesporial material. If this

conclusion is correct, aposporous gametophytes may sometimes arise. (Rightly or wrongly, it has been contradicted by BERGMAN.)

Consequently FAGERLIND would tell us about *Archieracium*: This subgenus shows simultaneously normal sexuality and apomixis. The type of apomixis is agamospermy, since the propagation is by seeds, and these are formed by that part-process of agamospermy which I call agamogony. Agamogony takes place by means of somatic apospory (GENTCHEFF), generative apospory (ROSENBERG, BERGMAN, GUSTAFSSON), semi-apospory (GUSTAFSSON), diplospory (BERGMAN). — EDMAN (and the present writer) would express the state of things quite simply so: *Archieracium* shows normal sexuality as well as agamospermy. A gametophyte is formed, either by means of apospory (GENTCHEFF) or diplospory (ROSENBERG, BERGMAN, GUSTAFSSON). The first division of the EMC shows various transition stages from a pure meiosis to a clean mitosis. — Which terminology is the simpler and at the same time the more adequate? To me the answer is unhesitatingly: That of EDMAN!

Take another instance. The mode of embryo development of an agamospermous plant has been settled. No nucellar embryony is present, and no embryo-sacs develop from chalazal or otherwise somatic cells. Unfortunately the character of meiosis or its substitute has not been fully established. Consequently FAGERLIND does not know whether the gametophyte is formed by generative apospory, semi-apospory, diplospory or a combination of these processes. EDMAN (and the present writer) would say: Though manifest meiotic stages are missing in the material available, we can safely conclude that the unreduced gametophyte is formed by diplospory.

A third instance. Apomictic pteridophytes — like the corresponding sexuals — still show an alternation of generations: the sporophyte gives rise to a gametophyte (prothallium), the gametophyte in its turn to a sporophyte, and the two generations are free from each other. The sporangia consist (1) of somatic cells forming sporangium walls and stalks and (2) of enclosed spore mother-cells. These two types of tissues are distinctly separated, no transitions existing between them. The prothallia are formed by spores which contain the reduced or the unreduced chromosome number, or they are formed from cells outside the sporial region. In the latter case all morphologists agree upon the denotation of apospory (which is somatic in character, according to FAGERLIND). In the case of prothallia formed from unreduced spores, FAGERLIND would say: Unfortunately we know nothing about

the course of meiosis in apomictic ferns; consequently I cannot tell you whether they show generative apospory, semi-apospory or diplospory. — EDMAN (and the present writer) at once exclaim: The matter is quite simple. The prothallium is formed by diplospory.

When we turn to the ferns, it immediately becomes evident that a sharp border-line separates the cases where gametophytes arise from extra-sporial and from sporial tissue. Not only is FAGERLIND's altered definition of the term diplospory improper, but his way of defining different processes by the course of the first division makes the terminology rather clumsy.

New sporophytes are formed by the autonomous development either of egg-cells or of other cells within the gametophyte. In the former instance we speak of *parthenogenesis*, in the latter of *apogamety* (apogamy). No new terms were introduced in 1940 for these two phenomena, which are specially manifest and distinct among ferns.

This short survey shows that the terminology which has crystallized out after forty years of debates and discussions, and which should be generally adopted, is entirely built upon WINKLER's and EDMAN's distinctions. FAGERLIND's attempts to create a new system of terms and definitions must be rejected, since they cause an unnecessary complication, besides being partially incorrect.

The following schemes may illustrate the different processes:

I. *Amixis* — occurs in organisms which lack sexual differentiation and fertilization.

II. *Mixis* — occurs in all higher organisms. Gamete formation combined with fertilization and alternation of nuclear phases.

$$\text{n-generation} \rightarrow \text{n-gametes} \xrightarrow{\text{Fertilization}} \text{2n-zygote} \xrightarrow{\text{Meiosis}} \text{n-spore} \rightarrow \text{n-generation} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ A$$

$$\underset{\text{Gametophyte}}{\text{n-generation} \rightarrow \text{n-gametes}} \xrightarrow{\text{Fertilization}} \text{2n-zygote} \xrightarrow{\text{Meiosis}} \underset{\text{Sporophyte}}{\text{2n-generation}} \rightarrow \text{n-spore} \rightarrow \text{n-generation} \quad \ldots\ B$$

III. *Apomixis* — occurs in organisms the relatives of which show sexual differentiation and alternation of nuclear phases. Implies therefore a secondary loss of the fertilization processes and the alternation of nuclear phases.

1. The reproduction is still by gametes.
Without alternation of generations (lower organisms).

Parthenogenesis
n-generation → n-gametes → n-generation ..... *C*

With alternation of generations (only ferns and phanerogams illustrated).

Parthenogenesis Apospory
n-gametophyte →n-gametes →n-sporophyte →n-gametophyte .... *D*
Prothallium ↑ Vegetative cells
Apogamety
Vegetative cells

Parthenogenesis Diplospory
2n-gametophyte →2n-gametes → 2n-sporophyte → 2n-spores →2n-gametophyte .... *E*
(Prothallium)
(Embryo-sac)
Apogamety Apospory
Vegetative cells Vegetative cells

D is restricted to ferns. In phanerogams the young sporophyte (*E*) is an embryo, parasiting on the mother individual.

2. The reproduction takes place by seeds but no alternation of generations occurs (phanerogams). *E*, when occurring among phanerogams, is together with *F* included under the term agamospermy.

Nucellar embryony
2n-individual→ 2n-ovules→2n-embryo →2n-individual ..... *F*
Nucellar cells

3. The reproduction occurs by cells lacking sex-character or by groups of cells, detached from the main-body (bulbils, etc.). (Vegetative multiplication.)

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NOTES ON SOME BOREAL POLYPLOIDS

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SINCE the first discovery (HAGERUP, 1927) of a certain correlation, in some plants, between polyploidy and geographical and ecological conditions, two new branches of botany, cyto-geography and cyto-ecology, have come into existence. As these sciences are so young, they are of course so far only in the initial stage; and the first groping attempts therefore require to be supplemented by wider and deeper study. This applies not least to the pioneer works (TISCHLER, LÖVE) based on statistic calculation, for the figures presented there all suffer from certain defects. Thus, if we consider a certain floristic area, the chromosome number of the species at hand has frequently been investigated in other regions, where a given species has often an entirely different chromosome number which cannot simply be transferred to other countries in calculations of a geographical kind (ARWIDSSON, 1938).

Another difficulty besetting cyto-geographical research is that no country has as yet been so thoroughly studied that the chromosome number of all species is known (LÖVE). The best known are the Northern countries, where the chromosome numbers of most species have been ascertained, so that the available statistics are already valuable. But it is a duty devolving on the botanists of every country to try to procure, in the course of time, a complete list of chromosome numbers, which should, as far as possible, be based on investigations of material from their *own* country.

To obtain a complete numerical material of this cyto-geographical kind is definitely within the limits of possibility, and such a material will indeed most likely be available within a measurable space of time. This little study is a modest contribution towards this end. I have realised once more how important it is for the botanists of different countries to help one another in collecting a material of species which are not easily accessible, and in this respect my special thanks are due to Dr. A. H. STENAR, of Östersund, for material of *Calypso bulbosa* and *Malaxis monophylla*. For my own part, I shall always be ready to collect for foreign colleagues.

The rest of my material has been collected in Denmark. Floral buds were, as a rule, fixed in the open in CARNOY's fluid; but in some cases the plants were planted in the Botanical Garden of the University of Copenhagen. Staining was carried out *ad modum* FEULGEN, which has always given excellent results.

Alisma plantago-aquatica L. n = 7 (Fig. 1). — This species is not particularly variable; nevertheless three different chromosome numbers

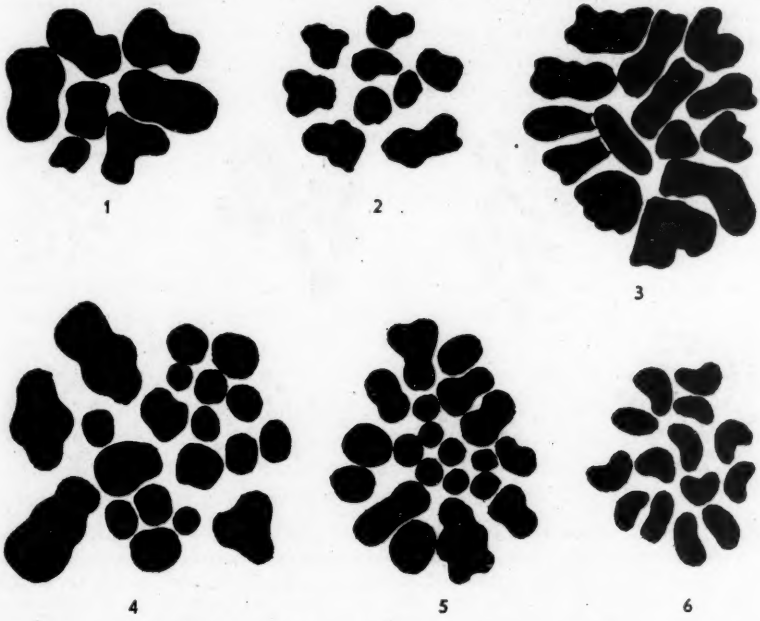

Fig. 1. *Alisma plantago-aquatica.* I metaphase. n = 7. — Fig. 2. *Echinodorus ranunculoides.* I metaphase. n = 9. — Fig. 3. *Alisma lanceolatum.* I metaphase. n = 2 × 7. — Fig. 4. *Epipactis atrorubens.* I metaphase. n = 20. — Fig. 5. *Epipactis palustris.* I metaphase. n = 20. — Fig. 6. *Calypso bulbosa.* I division in pollen. n = 14. — × 2880.

have been found for it, viz. n = 5, 6, and 7. Hence the possibility of geographical races in the different countries should be more closely investigated. My material was collected at Lyngby near Copenhagen, and meiosis showed 7 large chromosomes, which, however, are difficult to study because they always lie very close together. Meiosis may occur throughout the summer.

Alisma lanceolatum WITH. (= *A. stenophyllum* ASCH. et GR.).

$n = 2 \times 7$ (Fig. 3). — Of this rare species I had abundant material which had been collected on the island of Møn and planted in the Botanical Garden. Morphologically it is very much like *A. plantago-aquatica* and grows in similar localities. Its geographical distribution, however, is not so well known that it can be decided whether the two species are geographically different. But meiosis in June shows plainly 2×7 chromosomes of about the same size as in *A. plantago-aquatica*; thus *A. lanceolatum* is tetraploid in contrast to the closely allied common species, within which indeed polyploidy is not known.

Echinodorus ranunculoides (L.). $n = 9$ (Fig. 2). — The material was fixed in the Botanical Garden; the original locality of the plants is

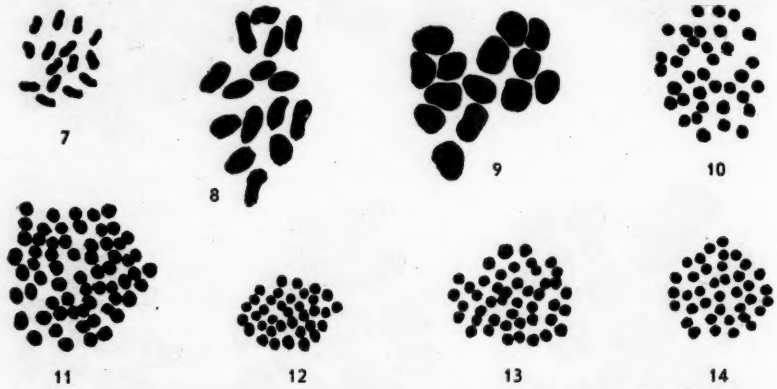

Fig. 7. *Malaxis monophylla.* I anaphase. $n = \pm 15$. — Fig. 8. *Spiranthes spiralis.* I metaphase. $n = 15$. — Fig. 9. *Hammarbya paludosa.* I metaphase in macrospore. $n = 14$. — Fig. 10. *Luzula pilosa.* I metaphase. $n = 6 \times 6$. — Fig. 11. *Cerastium triviale.* I anaphase. $n = 7 \times 9$. — Fig. 12. *Cerastium glutinosum.* I metaphase. $n = 4 \times 9$. — Fig. 13. *Cerastium brachypetalum.* I metaphase. $n = 5 \times 9$. — Fig. 14. *Eriophorum gracile.* I metaphase. $n = 38$. — × 2880.

unknown. At meiosis 9 large, angular chromosomes were found, of similar form to those of *Alisma.* Meiosis occurs at the beginning of the summer. The species is diploid.

Epipactis (*Helleborine*) *atrorubens* (HOFFM.). $n = 20$ (Fig. 4). — Planted in the Botanical Garden from the island of Møn, the only Danish locality of the species. Here it throve well. At meiosis 20 chromosomes of very different sizes were found.

Epipactis palustris L. $n = 20$ (Fig. 5). — This fairly common species was fixed in a bog near Slangerup in northern Sealand. It, too, has $n = 20$; and the chromosome complement is also reminiscent of

E. atrorubens. Meiosis occurs about a month before the flowering. Both species are perhaps diploid, 20 being probably the basic number of the genus.

Spiranthes spiralis (L.). n = 15 (Fig. 8). — Chief Veterinarian J. HARBOU fixed this rare plant from Thurø. At metaphase-I there occur 15 medium-sized chromosomes of about the same size. The species is diploid, and 15 is the basic number of the genus.

Hammarbya (*Malaxis*) *paludosa* (L.). n = 14 (Fig. 9). — I gathered this rare orchid in northern and western Jutland. During the development of the pollen it is, however, difficult to count the chromosomes because they are inclined to lump together. But I succeeded in finding some divisions in macrospores where it could be plainly seen that there are 14 medium-sized chromosomes.

Malaxis (*Achroanthes*) *monophylla* (L.). n = 15 (Fig. 7). — Dr. H. STENAR collected this rare species in central Sweden and kindly let me study his preparations. Meiosis was difficult to investigate because the chromosomes lump together; but as far as I could decide, n was = ± 15. For further details the reader is referred to STENAR's work (1937) on this plant.

Calypso bulbosa (L.). n = 14 (Fig. 6). — Dr. H. STENAR very readily lent me his preparations of this remarkable orchid, too, gathered in the north of Sweden. Meiosis occurs in the days around May 1st, and there are 14 fairly large crescent-shaped chromosomes at metaphase. The reader is referred to STENAR's work on *Calypso* (1940).

Luzula pilosa (L.). n = 6 × 6 (Fig. 10). — The species was gathered at Lyngby near Copenhagen in April. Meiosis occurs remarkably late, viz. 1—2 weeks before flowering. There are 36 small globular chromosomes at metaphase. The basic number of the genus is 6; so the species is markedly polyploid. The following numbers are known within the genus (see LÖVE's list, 1942 a): 6, 9, 12, 18, and 36. It should be ascertained whether the species has not other numbers.

Eriophorum gracile KOCH. n = 38 (Fig. 14). — This rare plant was gathered in Lyngby Bog near Copenhagen, where meiosis occurs in the course of May. At several metaphases I could distinctly count 38 chromosomes. In the other native species of the genus the numbers 27, 29, and 30 were found.

Arum maculatum L. n = 2 × 7 and 4 × 7 (Figs. 15 and 16). — This species is cytologically just as interesting as our other native Araceae. But supplementary investigations on this polymorphous species are needed from other countries. Plants from England had

n = 28 or 42 (MAUDE, 1940). I gathered plenty of wild-growing plants near Vordingborg in southern Sealand and easily counted n = 14. The species is fairly common in the southern parts of Denmark, but nearly all our wild plants belong to a form without spots on the leaves. In Denmark forms with spotted leaves are rarely found in nature; such plants were examined from the Botanical Garden in Copenhagen, though their original habitat was not known. The spotted individuals had 28 chromosomes at meiosis, which in addition were bigger than in our spontaneous plants. The basic number of the species is perhaps 7,

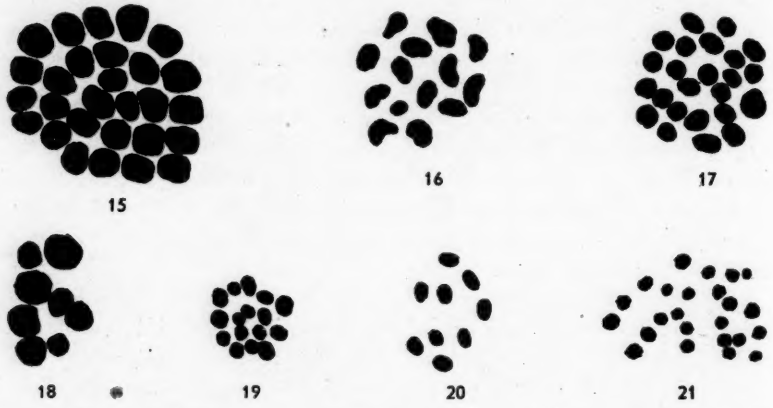

Fig. 15. *Arum maculatum*. I metaphase. n = 4 × 7. — Fig. 16. *Arum maculatum* (from Denmark). I metaphase. n = 2 × 7. — Fig. 17. *Monotropa hirsuta* var. *glabrescens*. I metaphase. n = 3 × 8. — Fig. 18. *Scabiosa canescens*. I metaphase. n = 8. — Fig. 19. *Linnaea americana*. I metaphase. n = 2 × 8. — Fig. 20. *Veronica scutellata*. I metaphase. n = 9. — Fig. 21. *Bidens radiata*. I metaphase. n = 2 × 12. — × 2880.

and this should be looked for in wild plants. Meiosis occurs in the beginning of May when the buds are just breaking through the surface of the ground. The different forms of the species seem to have a different geographical (and ecological?) distribution, which needs a more detailed charting.

Cerastium glutinosum FRIES. n = 4 × 9 (Fig. 12). — The species is so nearly allied to *C. semidecandrum* L. (n = 18) that its right to be called a separate species has often been questioned. But with some practice it is easily recognised habitually in natural conditions, and there are no transitional forms between the two species when they are growing together. I collected the species in southern Sealand (near Vording-

borg), and the plant then turned out to be tetraploid compared with *C. semidecandrum*, n being 36. Connected with this polyploidy are distinct ecological differences, *C. glutinosum* almost exclusively growing near the sea and rarely very far from it. The geographical conditions are no doubt also somewhat different, but this requires further investigation. Meiosis does not occur until May when the plants that have wintered begin to grow. The chromosomes are small, globular, and almost of the same size. The basic number of the genus is 9.

Cerastium brachypetalum DESPORTES. $n=5\times9$ (Fig. 13). — This pretty plant was gathered at Søndersø near Copenhagen. It is related to *C. glomeratum* ($n=36$). But the two species differ entirely in an ecological respect, since *C. brachypetalum* occurs spontaneously on dry uncultivated downs, while *C. glomeratum* is most frequently a weed in cultivated, not particularly dry places. A cytological investigation showed that the two species have a different chromosome number, since *C. brachypetalum* has $n=5\times9$. It should be ascertained whether there are numbers other than $n=4\times9$ within the somewhat polymorphous species *C. glomeratum*. Meiosis occurs at the end of April when the growth begins; the chromosomes are small, globular, and almost of the same size.

Cerastium triviale LINK (= *C. caespitosum* GILIB.). $n=7\times9$ (Fig. 11). — It is well known that this species is very polymorphous, not only morphologically but also ecologically. Some forms grow spontaneously on strand meadows and are annual, while others are perennial weeds on cultivated soil, etc. Any fairly large floristic handbook will furnish detailed information about this variation. ROHWEDER (1939) found that $n=72$ in one of the forms in Germany; and this high degree of polyploidy would seem to suggest that the species has several other numbers. I therefore fixed the buds of one of the usual forms from cultivated soil in Lyngby near Copenhagen and found that $n=63$. Perhaps several other numbers besides 7×9 and 8×9 may be found in this species, which ought to be subjected to a thorough investigation.

Monotropa hirsuta ROTH. (= *M. Hypopitys* L.). $n=3\times8$ (Fig. 17). — It is a well known fact that the species is somewhat polymorphous, and this has been more closely investigated by DOMIN (1915); but there is still great disagreement as to the systematic importance of the forms, since some authors only reckon with accidental variations without genetic value, while other investigators distinguish at least two species or subspecies, viz.

(1) *M. hypophegea* WALLROTH = *M. glabra* ROTH, which is glabrous and especially grows under *Fagus*, and

(2) *M. Hypopitys* L. = *M. hirsuta* ROTH., which is more or less hairy and occurs especially in coniferous forests.

However, all the distinguishing characters vary a great deal, and there exist forms intermediate between the two species, so that a cytological investigation would be of great interest. A good general view of the polymorphy is found in NORDHAGEN's Norwegian Flora (1940).

According to a written communication LÖVE was the first to investigate *M. glabra* from southern Sweden, and he found $2n = 16$. This curious figure obviously shows that 8 is the basic number of the genus. By way of comparison I examined the other species (*M. hirsuta*), which grows at Lyngby near Copenhagen. In order to find out the stages of the meiosis the exact localities were marked, and the following year the young shoots were dug up in June before they had broken through the surface. Meiosis occurs while the buds are still quite small and hidden between the leaves so that they are very difficult to find. At metaphase n was found to be 24, and *M. hirsuta* is thus hexaploid in contrast to *M. glabra*. It should be ascertained whether one of the other forms has $n = 16$?

The shape of the gymnaeceum showed that the plants examined by me belonged to the species *M. hirsuta*; but as only the external parts of the flower were hairy, it must be the var. *glabrescens* HOLMBOE. This will perhaps prove to be a hybrid between the two above-mentioned main species?

Thus the cytological conditions support the view that the two above-mentioned forms of *Monotropa* are separate species which differ both morphologically, ecologically, and in size. It should be investigated whether they also differ in geographical distribution.

Scabiosa canescens W. et K. (= *S. suaveolens* DESF.). $n = 8$ (Fig. 18). — This southern species has been planted in the Botanical Garden from N.W. Sealand. Meiosis occurs at the beginning of July, and $n = 8$ as in many other species of the genus. Thus the plant is diploid.

Veronica scutellata L. $n = 9$ (Fig. 20). — The species is somewhat variable. I examined both a robust and a slender form from Lolland, but both had $n = 9$ just like several other diploid species in the large genus.

Bidens radiata THUILL. $n = 2 \times 12$ (Fig. 21). — This rare plant has been planted in the Botanical Garden from its habitats in North

Sealand. Meiosis occurs throughout the summer and there are 24 chromosomes, as in its relative *B. tripartita*. Both species are tetraploid, the basic number of the genus being 12.

Linnaea americana FORBES. $n = 2 \times 8$ (Fig. 19). — This species differs very little from the European *L. borealis* as far as the external morphological features are concerned. On the other hand, the two species seem to differ somewhat ecologically: the American species thrives well in the Botanical Garden in Copenhagen, where it has been raised from seeds that have come direct from Canada, whereas *L. borealis* as a rule dies in the course of a brief space, even though it receives the same treatment in the garden. In Greenland *L. americana* grows in some few places in a low scrub of arctic bushes (*Empetrum*, *Salix glauca*, and *Vaccinium uliginosum*) and is found as far north as Disco (70°), where it thrives well and flowers profusely. Altogether it is somewhat larger and more robust than *L. borealis*; and as a matter of fact a cytological investigation shows that it is tetraploid with $n = 16$. Whether *L. borealis* is diploid I hope soon to be able to decide, but it is rare in Denmark and difficult to procure. Meiosis occurs in May when the flower-bud is about 2 mm long and is beginning to show outside the surrounding leaves.

SUMMARY.

Meiosis has been investigated from a cyto-ecological point of view, and the chromosome number has been determined for the following species:

	n =
Alisma plantago-aquatica	7
» *lanceolatum*	2×7
Echinodorus ranunculoides	9
Epipactis atrorubens	20
» *palustris*	20
Spiranthes spiralis	15
Hammarbya paludosa	14
Malaxis monophylla	± 15
Calypso bulbosa	14
Luzula pilosa	6×6
Eriophorum gracile	38
Arum maculatum	2×7 and 4×7
Cerastium glutinosum	4×9
» *brachypetalum*	5×9

Cerastium triviale	7 × 9
Monotropa hirsuta (= *M. Hypopitys*)	3 × 8
Scabiosa canescens	8
Veronica scutellata	9
Bidens radiata	2 × 12
Linnaea americana	2 × 8

Special interest attaches to the polyploid species: *Alisma lanceolatum*, *Luzula pilosa*, *Arum maculatum*, *Cerastium* sp., *Monotropa*, *Linnaea americana*, and *Bidens radiata*.

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ON THE NORMAL OCCURRENCE OF CHROMOSOME DOUBLINGS IN SECOND-YEAR ROOT TIPS OF SUGAR BEETS

BY *ALBERT LEVAN*
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IN his classical paper of 1916 WINKLER points out that in its normal ontogeny *Solanum lycopersicum* makes use of polyploidy at the differentiation of certain types of tissues, such as the marrow, the endodermis of the root, and the collenchyma of the stalk. He cites several instances of the same phenomenon (l. c., pp. 480—490), and many other cases have become known since then. Recent instances are studied by GEITLER and co-workers both in plants (*Sauromatum*; GRAFL, 1939) and animals (*Gerris*; GEITLER, 1939).

The occurrence of tissues or parts of tissues with polyploid chromosome number may be inferred from mere observations of the nuclear and cellular size. Or a characteristic heterochromatic chromosome may be visible during the resting stage, from which the stage of polyploidy may be determined by estimating the number of such chromosomes present. More seldom (*Spinacia, Cannabis*) the number of chromosomes may be directly counted in the mitoses occurring in the old tissues. Cells which normally have ceased to divide may be stimulated into mitosis by wounding (GRAFL, l. c.) or by treatment with growth substances (LEVAN, 1939). In the latter case it is probable that the growth substances are to some extent the cause of the polyploidy by the c-tumour growth they induce in the root cortex. But no doubt the old cortex of untreated roots also normally contains polyploid nuclei. Spontaneous polyploidy may also be revealed by bringing an old specialized tissue back into a meristematic condition. This was the case in the regeneration experiments on decapitated *Nicotiana* by GREENLEAF (1938). During my routine work on the control of the polyploid sugar beet material, cultivated at Hilleshög for breeding purposes, I came across the new instance of spontaneous somatic polyploidy described below.

The somatic chromosomes of the sugar beets are usually studied in

the young roots of the germinating seed. Last autumn (1942), however, about 1500 fullgrown beet roots were harvested and stored for chromosome examination. During January, 1943, the roots were brought into warmer temperature (18° C) and were covered with moist canvas. In a few days a great many root tips appeared in the neighbourhood of the two »root furrows» of each root. The root tips were fixed in Navashin and were usually found to contain an abundance of very clear mitoses. A close study of the slides (each slide containing cross sections of 6 roots) showed that polyploid sectors and entire polyploid roots occurred much more frequently than is the case in the root tips of the seed. The material contained F_1 beets from crosses between diploids and tetraploids and progenies of diploid and tetraploid controls. The chromosome numbers of the material will be discussed in another connection by RASMUSSON and LEVAN. On this occasion only the following rough summary of the chromosome numbers will be given:

	Total investigated plants	Number of plants with observed doublings	% Doublings
Diploids	342	63	18,4
Triploids	399	39	9,8
Tetraploids	729	39	5,4
Total	1470	141	9,6

The first column gives the total number of studied plants, the second column gives the number of plants in which polyploid mitoses were observed. As only 6 root tips were studied in each plant, it is evident that the second column furnishes only a random estimate of the frequency of doublings. If sufficient material were studied, doublings would probably be met with in every beet plant. The frequency of doublings given in the third column is reliable as a random sample. Doublings evidently occur most frequently in diploids and more rarely in triploids and tetraploids. This may be connected with the fall in viability which undoubtedly occurs from tetraploid to octoploid cells. The octoploid cells originating in a tetraploid plant may more easily get lost than the tetraploid cells of a diploid plant.

A detailed examination of the slides from the plants with doublings shows that in all cases the normal diploid chromosome number was predominant. Very often five roots were normal diploid while one root was tetraploid or contained a tetraploid sector. An arrangement of the

doubled material according to the frequency of doubled cells gives the following table:

	2x	3x	4x
One or two roots entirely doubled	76,2	46,1	33,3 %
Only a sector of one root doubled	23,8	53,9	66,7 »

Thus, this also indicates a falling frequency of doublings from 2x to 4x. In one case a tetraploid root contained a 16-ploid sector, in which 144

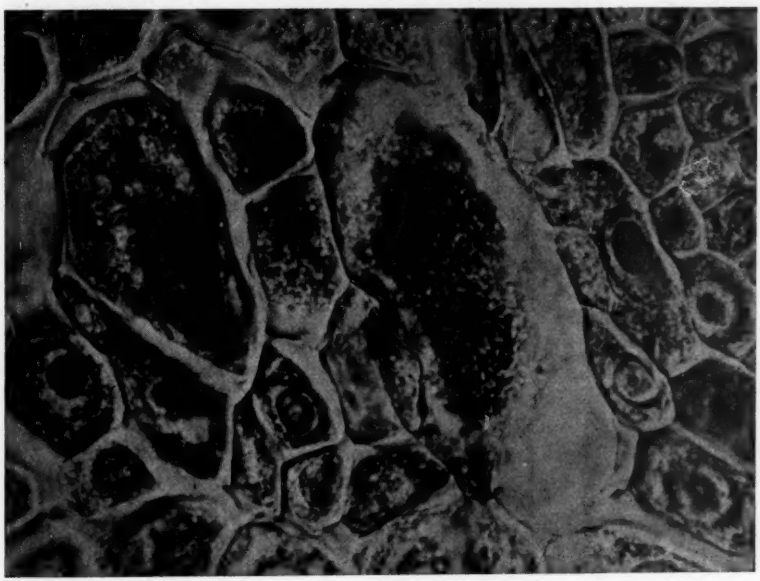

Fig. 1. A 16-ploid sector in a root tip of a tetraploid sugar beet plant. — × 1250.

chromosomes could be clearly counted (Fig. 1). This is the only instance in which two doublings following each other in the same cell could be demonstrated, even though this probably occurred rather often judging from the cell size.

In order to get a comparison for the conditions of the root tips of young seedlings an examination was made of the records of the routine chromosome determinations in beet seedlings made during the summer of 1943. The following data were at hand:

	Total investigated plants	Number of plants with observed doublings	% Doublings
Haploid	1	0	—
Diploids	1703	4	0,24
Triploids	1130	3	0,27
Tetraploids	762	1	0,13
Pentaploids	2	0	—
Hexaploids	9	0	—
Total	3607	8	0,22

Thus, there is a striking difference in the frequency of chromosome doublings. While the doublings in roots from old beets occur in about 10 % of the studied material, the corresponding percentage in roots of beet seedlings is only 0,2 %.

Concerning the nature of the chromosome doublings found in the second-year root tips, they may be regarded as samples of the doublings normally occurring during the development of the sugar beet plant as well as probably in every plant species. The work of BERGER (1938), GEITLER (l. c.), GENTCHEFF and GUSTAFSSON (1939) as well as the experimental attack on the problem by myself (LEVAN, l. c.) has demonstrated that this type of polyploidy originates by endomitosis. This process seems to be started as soon as the cells attain a certain volume, either in the course of the normal growth of the tissue or induced by external agents (the c-tumour-reaction). It is easily understandable that the secondary root meristems of the old beets may contain cells which during their development have undergone endomitosis.

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THE X-RAY RESISTANCE OF DORMANT SEEDS IN SOME AGRICULTURAL PLANTS

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IN the year 1940 a special line of plant-breeding research was begun at the Swedish Seed Association, Svalöf. Mutations, up to that time considered to have no importance for plant-breeding, were induced in barley and wheat during the years 1933—1939 to such a great number and with such a striking appearance that the Director of the Seed Association, Prof. Dr. Å. ÅKERMAN, considered it necessary to embark on a long-run program for the methodical induction of new and valuable mutations. A special fund, granted by A.-B. Saltsjöqvarn, Stockholm, made it possible to carry out a series of practical and theoretical investigations. Results of interest to plant-breeding have already appeared and have partially been published. In this paper only one problem will be taken up, but a rather important one, namely the response of different kinds of seeds to increasing X-ray dosages. It is evident that for a proper induction of mutations in plant-breeding work we must know the exact dosages to be applied.

MATERIAL AND METHODS.

Dormant seeds, previously stored for a short time under the usual conditions, were X-rayed at the Radiophysics Institute, Stockholm (Director: Prof. Dr. R. SIEVERT), under the supervision of Dr. A. FORSSBERG. The methods used have been repeatedly published. The voltage was most frequently 176.000 volts, the current 4 milliamperes. Only the intensity (r/min.) varied somewhat in different years and series (usually from 1.000 to 3.000 r/min.). In the case of large-sized seed material (for instance dwarf-beans) the focal distance was made greater and the intensity lower (in the case mentioned 275 r/min.). Filters of copper and paper having a thickness of 0,05 and 1 mm. were used. For further details the reader is referred to a paper by FRÖIER in 1941.

Seeds of the following species were treated:

C a n n a b a c e a e: hemp (*Cannabis sativa*),

Cruciferae: rape (*Brassica napus*), swedes (*Brassica napus* var. *napobrassica*), mustard (*Sinapis alba*),

Papaveraceae: poppy (*Papaver somniferum*),

Leguminosae: white-clover (*Trifolium repens*), peas (*Pisum sativum*), dwarf-beans (*Phaseolus vulgaris*), lupines (*Lupinus luteus*), soy-beans (*Glycine Soya*),

Linaceae: flax (*Linum usitatissimum*),

Compositae: sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*),

Graminaceae: barley (*Hordeum vulgare* and *distichum*), wheat (*Triticum vulgare*), oats (*Avena sativa*), rye (*Secale cereale*), meadow-grass (*Poa pratensis*).

The seed material was handed over to the respective departments of the Seed Association immediately after irradiation and was sown together with other material of the same species under field-conditions (except in the case of *Papaver*, *Trifolium* and *Poa*; v. below). Laboratory experiments were also carried out to a great extent, but these will not be dealt with here [cf. FRÖIER and GUSTAFSSON, 1941, 1944 (in preparation)]. Nor will investigations concerning the influence of high water content, chemicals, etc. be included in this paper.

The susceptibility of different kinds of seeds was determined by the number of plants surviving at the time of full growth or ripeness and producing at least one seed per plant. In most instances the immediate germination was also recorded, as was the germination in the Laboratory of the Seed Control Department. I will rest content here, however, with discussing the figures concerning the final plant-survival in field experiments, those being the only figures of importance for plant-breeding work. The mutation results will be described in a series of papers later on, in co-operation with the heads of the different departments.

RESULTS.

Cannabaceae.

The strain of *hemp* used was »Schurig». Data from 1941 and 1942 are available. The dosages in 1941 were 5.000, 15.000, 25.000 and 35.000 r, in 1942 5.000, 7.500 and 10.000 r units. The results are listed on p. 167, above.

It is evident that under field-conditions the critical dosage lies somewhere between 5.000 and 10.000 r, apparently in the region of 7.000—8.000 r.

	1941		1942	
	Seeds	Full-grown plants	Seeds	Full-grown plants
Control ...	75	48 %	75	56 %
5.000 r ..	150	41 »	75	47 »
7.500 » ..	—	—	150	15 »
10.000 » ..	—	—	300	1 »
15.000 » ..	300	1 »	—	—
25.000 » ..	450	0 »	—	—
35.000 » ..	600	0 »	—	—

Cruciferae.

Rape. — The material belonged to the strain »Regina» (a spring form), cultivated generally in Sweden. Unfortunately, the young seedlings were so severely attacked by flea-beetles in 1942 that no plants survived. It is clear that dry seeds of rape stand a dosage of 15.000 r without any harm at all; on the contrary, the germination percentage seemed to increase, at least in the year 1941. The control (75 seeds) germinated in 1941 and gave full-grown plants to 44 %, while the corresponding figures at 5.000, 15.000, 25.000 and 35.000 r (75, 75, 300 and 585 seeds respectively) were 68, 63, 26 and 4 %. In 1943 the seed-material, consisting of 100 seeds in each series, was given 25.000 and 35.000 r. The control produced 22 % ripe plants. With 25.000 r 43 % and with 35.000 r 16 % ripe plants were obtained. If the data of these two years are reliable, the critical dosage should lie above 30.000 r units.

Swedes. — In 1942 the seeds of the Svalöf strain »Swedish yellow 016» were sown individually in 15 m. long rows at a distance of 25 cm. from each other. In 1943, when much higher dosages were applied, it was thought advisable to sow five seeds together in each hole in order to avoid too great gaps in the cultures. It was found, however, that the germination was also very good at the highest dosages. Therefore the rows had to be singled. The percentages of survival, given below, consequently relate to the *number of groups* which at the time of singling contained at least one seedling that was still alive at harvest-time. No doubt can exist that the critical dosage lies well above 90.000 r, although some plants treated with 75.000 and 90.000 r look very weak and frail. (Table, see p. 168, above.)

X-rayed seeds stored for one year after the irradiation gave an inferior survival, and most full-grown plants appeared deficient even after 20.000—25.000 r.

Mustard. — Equally astonishing is the very high resistance of the oily seeds belonging to this species (a Svalöf strain). In fact, they stood

Swedes.

	1942		1943 [1]	
	Seeds	Full-grown plants	Seeds	Full-grown plants
Control I ..	240	52 %	240	85 %
» II ..	240	47 »	240	54 »
2.500 r ..	240	37 »	—	—
5.000 » ..	240	38 »	—	—
7.500 » ..	240	37 »	—	—
10.000 » ..	240	33 »	—	—
12.500 » ..	240	37 »	—	—
15.000 » ..	240	33 »	—	—
17.500 » ..	240	34 »	—	—
20.000 » ..	240	32 »	—	—
22.500 » ..	240	30 »	—	—
25.000 » ..	240	35 »	—	—
30.000 » ..	—	—	240	91 »
40.000 » ..	—	—	240	66 »
50.000 » ..	—	—	240	54 »
60.000 » ..	—	—	240	73 »
75.000 » ..	—	—	240	71 »
90.000 » ..	—	—	240	60 »

a dose of at least 100.000 r and in 1941 germinated better than the control also at 35.000 r. In order to lower the germination sufficiently for mutation purposes a dosage of at least 110.000 r should be applied. A superior method of handling this plants is however to have the seeds stored after the irradiation for a year or two before the sowing. In such experiments the X-ray effects are enhanced, and the germination and growth are visibly inferior to those of the controls or of the newly irradiated material. Both methods are now tested at Svalöf.

	1941		1942		1943	
	Seeds	Full-grown plants	Seeds	Full-grown plants	Seeds	Full-grown plants
Control ..	75	28 %	75	89 %	100	64 %
5.000 r ..	75	36 »	—	—	—	—
10.000 » ..	75	49 »	—	—	—	—
25.000 » ..	300	55 »	75	78 »	100	50 »
35.000 » ..	600	61 »	75	73 »	—	—
40.000 » ..	—	—	—	—	100	57 »
45.000 » ..	—	—	300	70 »	—	—
55.000 » ..	—	—	450	80 »	100	55 »
70.000 » ..	—	—	—	—	100	43 »
85.000 » ..	—	—	—	—	100	62 »
92.000 » ..	—	—	—	—	100	35 »

[1] For the year 1943, see the text.

Papaveraceae.

Poppy. — The strain used was »Mahndorfer». The dosages were in 1941: 2.500, 5.000, 10.000, 15.000, 20.000, 30.000 r, in 1942: 5.000, 7.500, 10.000, 15.000 r. Owing to the smallness of the seeds it was necessary to sow them in boxes of soil. The survival percentages were ?, 44, 2, 0, 0 in 1941 and 45, 45, 36, 23 in 1942. The corresponding control figure in 1942 was 45 %. (In 1941 the control was discarded immediately after germination, which amounted to about 100 %.) The number of seeds was 1.000 in each series. The critical dosage lies at 10.000 to 20.000 r (?). The two series of experiments ran somewhat differently, this being the reason that a precise figure is difficult to state.

In poppy, as in soy-beans, mortality does not set in until the plants have passed the seedling-stage, i. e. at a later stage than in cereals, where the plumules of severely damaged seedlings cannot break the soil surface.

Leguminosae.

White-clover. — The X-rayed seeds of this species (a self-fertile strain of Mossö) were planted for germination in a different manner. Owing to practical difficulties they were not sown directly in the field but germinated in gravel indoors, the layer above the seeds being $1^1/_2$ cm. thick. This makes the seedlings less damaged by the X-ray treatment. Nevertheless, it is surprising that even after a dosage of 30.000 r they germinate and shoot through the gravel to approximately the same extent as the control (this in marked contrast to meadow-grass; cf. below). Until further experiments have been carried out, white-clover should not be regarded as especially resistant, although the seeds are no doubt less sensitive than, for instance, those of soy-beans. A slight increase in germination at 10.000 up to 20.000 r is recognizable, but has hardly any significance.

		Germination 1941						
		13/8	15/8	17/8	19/8	21/8	24/8	
Control	100 seeds	19	31	12	3	1	—	66 %
2.500 r	» »	19	38	4	—	—	—	61 »
5.000 »	» »	22	30	9	1	1	—	63 »
10.000 »	» »	24	29	15	2	2	—	72 »
15.000 »	» »	16	39	11	4	1	1	72 »
20.000 »	» »	14	44	13	3	1	1	76 »
25.000 »	» »	10	32	14	4	3	—	63 »
30.000 »	» »	11	45	7	5	2	2	72 »

Peas. — The seeds belonged to the strain »Svalöf 03023». The critical point lies at 5.000—10.000 r. The results are given in the table on p. 172, above. All seedlings, germinated in the 10.000 r series (and above), were very small and weak.

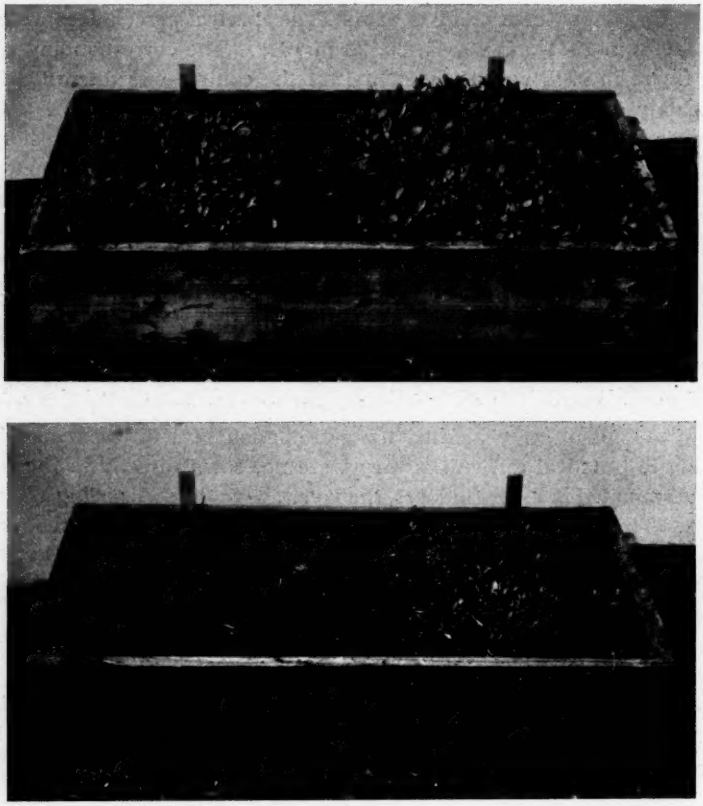

Fig. 1. The influence of increasing X-ray dosage on the germination and growth of poppy. — Above from right to left: control and 2.500 r, below from right to left: 5.000 and 10.000 r. — By courtesy of Dr. G. ANDERSSON, Svalöf. 1941.

Dwarf-beans. — The material used belonged to one line, »G. Eriksson 1». In 1941 the treatments were 5.000, 10.000, 15.000, 20.000, 25.000, 30.000, 35.000 and 40.000 r. No seeds germinated and grew in field-cultures above 10.000 r. The control gave 88 % full-grown plants, the 5.000 r and the 10.000 r treatment gave 89 and 15 % respectively.

75 seeds were sown in each series. In 1942 the control gave 93 % plants. At 5.000 r (75 seeds) the percentage was 85, at 7.500 r (150 seeds) 72 % and at 10.000 r (150 seeds) 29 %. Evidently the critical point lies at approximately 10.000 r.

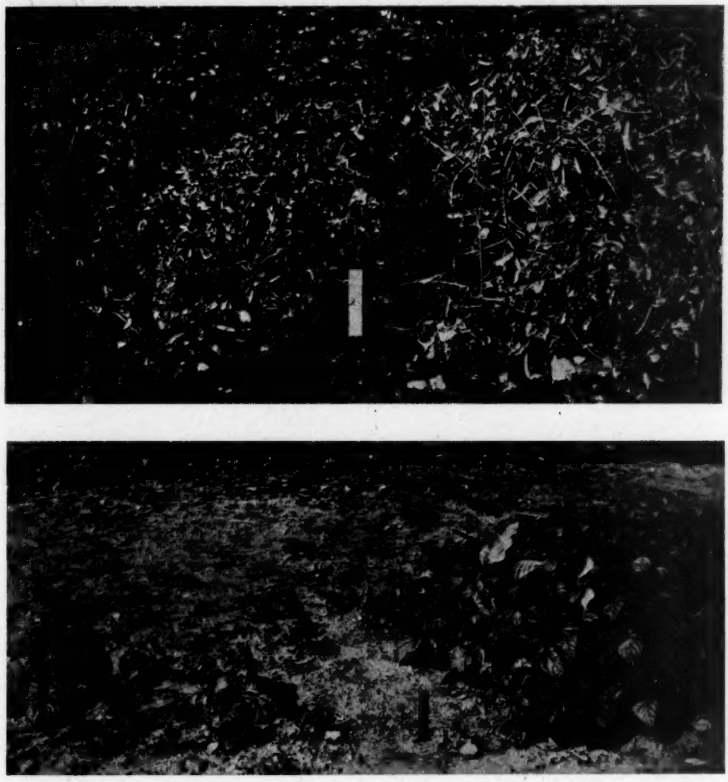

Fig. 2. The influence of increasing X-ray dosage on the germination and growth of peas (above) and dwarf-beans (below). — Above to the right, control, to the left, 5.000 r. Below to the right, 5.000 r, to the left, 10.000 r. — By courtesy of Dr. I. GRANHALL. 1941.

Lupines. — In 1942 and 1943 the strain »Yellow sweet» was X-rayed, in 1943 also »Yellow bitter, 42/195». Unfortunately the dosages given in 1943 were too high. The critical point lies somewhere in the region of 15.000 r. The results were (1) »Yellow sweet», 1942: Control — 54 %, 2.500 r — 16 %, 5.000 r — 17 %, 7.500 r — 15 %, 10.000 r —

Peas.

	1941		1942	
	Seeds	Full-grown plants	Seeds	Full-grown plants
Control ..	60	72 %	45	93 %
5.000 r ..	150	51 »	45	44 »
10.000 » ..	150	19 »	45	2 »
15.000 » ..	150	15 »	45	2 »
20.000 » ..	150	8 »	45	2 »
25.000 » ..	150	3 »	45	0 »
30.000 » ..	150	0 »	45	0 »
35.000 » ..	150	0 »	45	0 »

17 %, 12.500 r — 6 %, 15.000 r — 9 %, 17.500 r — 3 %, 20.000 r — 1 %, 22.500 r — 2 %, 25.000 r — 1 %, 1943: Control — 49 %, 17.500 r — 1 %, 20.000 r — 2 %, 22.500 r — 2 %, (2) »Yellow bitter»: Control — 86 %, 17.500 r — 7 %, 20.000 r — 2 %, 22.500 r — 5 %. The number of seeds X-rayed varied from 190 to 208 in the different series.

Soy-beans. — Three strains were tested of this species: »Manitoba Brown», »Altonagaard» and »Sv. 38/66». It was found in several experiments that seeds did not stand higher dosages than 10.000 r; in fact the critical point seems to lie in the 5.000—7.500 r range. In 1941 the dosage series was 5.000, 10.000, 15.000, 20.000, 25.000 and 30.000 r. 125 seeds of each strain were treated with each dosage. The percentage of harvested plants treated at 5.000 r amounted in »Manitoba Brown» to 26 and in »Altonagaard» to 8 %. No seed-bearing plant of »Sv. 38/66» was obtained. At no higher dosage than 5.000 r were seeds produced. — In 1942 the weather-conditions at Svalöf were very harsh for soy-bean cultivation, and so neither the control nor the X_1 plants got sufficient sunshine and drought for their ripening. In this year the dosages given were 2.500, 5.000, 7.500 and 10.000 r. The same three strains as in 1941 were treated. In each series and variety 200 seeds were irradiated and sown. The percentages of well-developed plants are given in the following table.

	Control	2.500 r	5.000 r	7.500 r	10.000 r
Manitoba Brown	30 %	37 %	28 %	17 %	2 %
Altonagaard	48 »	50 »	28 »	5 »	3 »
Sv. 38/66	48 »	38 »	32 »	14 »	4 »

Linaceae.

The two kinds of *flax* (fibre and oil flax) react in the same way to X-rays. The strains used were »Herkules» and »Svalöf 01000». The results are immediately to be seen from the table on p. 174. In 1941 the

critical dosage seemed to lie about 45.000 r, but in 1942 it appeared to be higher. It ought to be 40.000—60.000 r. In flax as well as in rape and mustard it was found that material stored for one year germinated and

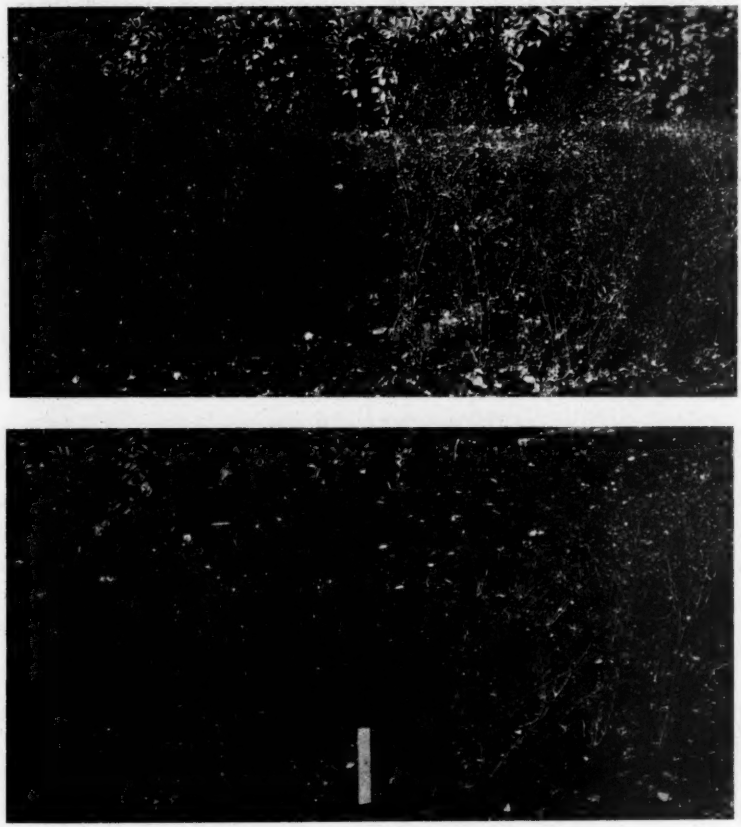

Fig. 3. The influence of increasing X-ray dosage on the germination and growth of fibre flax. — Above to the left, 15.000 r, to the right, 25.000 r. Below to the left, 25.000 r, to the right, 35.000 r. — By courtesy of Dr. I. GRANHALL. 1941.

grew very poorly. Such treatment of the seeds will probably prove to be of importance in plant-breeding.

Compositae.

Sun-flower. — In 1941 as well as in 1942 the dosages given were 5.000, 10.000, 15.000 and 20.000 r, in 1941 also 25.000, 30.000, 35.000

	1941		1942	
	Seeds	Full-grown plants	Seeds	Full-grown plants
		Fibre flax		
Control ..	150	77 %	150	89 %
5.000 r ..	150	71 »	—	—
15.000 » ..	300	67 »	—	—
25.000 » ..	750	57 »	300	88 »
35.000 » ..	1200	39 »	300	64 »
45.000 » ..	—	—	900	52 »
		Oil flax		
Control ..	150	58 %	150	82 %
5.000 r ..	150	47 »	—	—
15.000 » ..	300	41 »	—	—
25.000 » ..	750	33 »	300	65 »
35.000 » ..	840	14 »	300	95 »
45.000 » ..	—	—	900	49 »

and 40.000 r. These latter dosages killed the seeds completely so that the seedlings were not able to penetrate the soil-surface. But even after treatment with the lowest dosage the germination was imperfect and only occasional individuals (5—6 %) were living at the time of harvest. 150 to 200 seeds were rayed in each series. In the controls 70—100 % of the seeds germinated. Owing to lack of space the control specimens were discarded at an early stage, so that no comparable data on the number of surviving plants are available, but we know from other material that the survival is much higher than in the X-ray treated series. Therefore, if the results of these two years can be generalized, the critical dosage lies at about 5.000 r or perhaps lower.

Safflower. — The work with this plant is made rather difficult at Svalöf owing to the capricious ripening and germination of the seeds. In 1942, however, the seeds were well developed and germinated in the controls to 72 %. The dosages applied were 2.500, 5.000, 10.000, 15.000 and 20.000 r, 500 seeds being treated in each series. In the first two series the number of surviving plants amounted to 30 and 19 %, for the highest three dosages it was nil. Consequently, the critical dosage should lie between 5.000 and 7.500 r.

Graminaceae.

In several papers the dosage relationships have been described for seeds of barley, wheat and oats. In a paper to be published shortly by FRÖIER and GUSTAFSSON the behaviour of different strains (naked and covered barley, six-row and two-row barley, covered and decovered

oats, etc.) will be discussed in detail. As to barley, six-row and naked varieties are somewhat more sensitive to X-rays than two-row strains, the critical dosage for which lies at about 20.000 r in field-cultures (see GUSTAFSSON, 1942, Table 1). The corresponding dosage for summer and winter wheat is about 15.000—20.000 r, i. e. lower than was reported in laboratory experiments by FRÖIER and GUSTAFSSON in 1941. The critical dosage for normal hexaploid oats is about 15.000 r. Consequently, no striking difference between the three cereal species is to be found, although the number of cytological disturbances is greatly increased (about three times) in hexaploid as compared with diploid varieties of the same genus.

Rye. — The strain tested was »Kungsråg II» (King's rye II), which is a winter type. The dosages used were 5.000, 10.000, 15.000 and 20.000 r, 400, 400, 800 and 800 seeds being rayed respectively. The number of over-wintered plants amounted to 42, 22, 4 and 0 %. The control gave 51 % surviving plants. Apparently the critical dosage lies at approximately 12.500 r.

Meadow-grass. — The seeds, belonging to the apomictic variety »Fylking», were for practical reasons sown indoors in boxes with a thin layer of soil above the seed surface (1941). The slow germination of the control also necessitated a long period of observation. The critical dosage lies in this experiment at 15.000 r. Consequently it should be somewhat lower in field-cultures.

		Germination after		
		29 days	33 days	41 days
Control I	200 seeds	62 %	63 %	64 %
» II	» »	44 »	44 »	48 »
2.500 r	» »	57 »	57 »	61 »
5.000 »	» »	46 »	50 »	55 »
10.000 »	» »	46 »	50 »	53 »
15.000 »	» »	4 »	7 »	12 »
20.000 »	» »	1 »	3 »	5 »
25.000 »	» »	0 »	0 »	0 »
30.000 »	» »	0 »	0 »	0 »
40.000 »	» »	0 »	0 »	0 »
50.000 »	» »	0 »	0 »	0 »

DISCUSSION.

This study aimed at determining the most suitable X-ray dosages for mutation experiments in a series of cultivated plants. A glance at Table 1 immediately shows the striking differences occurring. Compare,

for instance, *Brassica napus* var. *napobrassica* and *Sinapis alba* with *Glycine Soya* or *Helianthus annuus*, the seeds of the first-mentioned two species standing dosages of at least 90.000—100.000 r, the latter two species no more than 7.500 r!

The different plant-families seem to react to X-rays in a distinct way. The three Cruciferae-varieties are highly resistant, although the resistance is less pronounced in the case of rape. The species of

TABLE 1. *The critical X-ray dosages in various agricultural plants.*

	Chromosome number	1000-grain weight	Critical dosage
Cannabis sativa	2n = 20	16,5 gr.	7.500 r
Brassica napus	2n = 38	4,0 »	> 30.000 »
» » var. *napobrassica*	2n = 38	2,5 »	> 90.000 »
Sinapis alba	2n = 18	8,5 »	> 92.000 »
Papaver somniferum	2n = 22	0,5 »	(± 15.000 »)[1]
Trifolium repens	2n = 16	0,65 »	(> 30.000 »)[1]
Pisum sativum	2n = 14	185 »	7.500 »
Phaseolus vulgaris	2n = 22	395 »	10.000 »
Lupinus luteus	2n = 24	125 »	15.000 »
Glycine Soya	2n = 40	140 »	7.500 »
Linum usitatissimum	2n = 32	4,8—9,0 »	40.000—60.000 »
Helianthus annuus	2n = 34	75 »	≦ 5.000 »
Carthamus tinctorius	2n = 24	30 »	7.500 »
Hordeum species	2n = 14	45 »	10.000—20.000 »
Triticum vulgare	2n = 42	45 »	15.000—20.000 »
Avena sativa	2n = 42	40 »	15.000—20.000 »
Secale cereale	2n = 14	35 »	12.500 »
Poa pratensis	2n = ± 72	0,3 »	(10.000—15.000 »)[1]

Leguminosae contrast strikingly, the critical dosage lying at about 7.500—10.000 r (the higher critical dosage in white-clover depends most probably on the mode of sowing). The five grass species, finally, behave rather similarly to one another, all of them standing about 15.000 r units. Varietal differences occur, however, but do not markedly alter the resistance.

It has been shown by a great many workers that the physiological state of the treated material highly influences the X-ray effect and thence the number of chromosome disturbances and mutations. The X-ray effect depends, for instance, on the metabolic activity, the degree of dormancy, the stage of mitosis (or meiosis) at the time of irradiation,

[1] Laboratory experiments.

etc. Of special importance is the hydration of the cell and nucleus. These results indicate that the physiological condition of the nucleus, or more specially the physiology of the chromosomes, is directly responsible for the rate of mutation, since the number of mutations in different material runs parallel to the number of chromosome disturbances, and these to the position of the critical dosage (GUSTAFSSON, 1940; GELIN, 1941). It is hardly possible to dispute that a seedling (or an X-ray treated tissue) will die when the chromosomes get so crushed that they cannot effect a regular mitosis. The amount of chromosome breakage and rearrangement is therefore less influenced by the number of actual hits upon the chromosomes than by the physiological properties of the irradiated cell and nucleus. Or, in other words, the number of *acting* ionizations is determined by the physiology of the cell.

An actual proof of this conclusion is afforded by the Cruciferae-species used in the experiments. The seeds are very small (the 1000-grain weight is less than 10 gram) and the radicula of the embryo lies rather exposed close to the testa. Since the voltage used amounted to 176.000 volts, the X-rays were extremely hard. They penetrate no doubt easily through the seeds. Nevertheless these resist dosages by which every cell, every nucleus and every chromosome is hit many times, and they germinate normally and give maturing plants lacking any trace of sterility. It was shown by FRÖIER, GELIN and GUSTAFSSON (1941) that almost all embryonal root-cells in dormant seeds of *Triticum* and *Avena* were so damaged by a dosage of 20.000 to 25.000 r that no subsequent mitosis was normal. Consequently, in the case of mustard and swedes the hits get inactivated in some manner and the chromosomes enter division as healthy as if no bombardment at all had occurred.

There is hardly any other factor influencing the resistance in these instances than the seed content of fats (eruca, arachin, behen and rapin acids), which in some way protect the chromosomes, prevent their breakage, or make the nuclei insensitive to irradiation. Supporting this explanation is the fact that the oily seeds of flax likewise stand rather high dosages, higher than starch- or protein-rich seeds of most other material, contradicting it that the seeds of sun-flower and safflower are greatly susceptible.

The writer is indebted to colleagues and friends at the Swedish Seed Association for valuable co-operation and discussions.

SUMMARY.

In order to find the most suitable X-ray dosages for altering genes and chromosomes in various cultivated plants, methodical irradiation experiments with dormant seeds have been carried out at the Swedish Seed Association, Svalöf, and the Radiophysics Institute, Stockholm. The critical dosages, which still produce a fair amount of viable X_1 offspring (field material) but simultaneously produce a high rate of mutations and chromosome alterations, are gathered in Table 1, to which the reader is referred.

Seeds of several oil-plants, as, for instance, those of the three Cruciferae-varieties *Brassica napus*, *Brassica napus* var. *napobrassica* and *Sinapis alba*, as well as those of *Linum usitatissimum*-lines, are highly resistant to X-rays, in contrast to seeds of Leguminosae- and Compositae-species. Seeds of grass-species are rather easily influenced but stand dosages up to 15.000 or 20.000 r. The chromosomes of the first-mentioned four oil-plants are thought to be rendered resistant to X-ray hits and ionizations by some physiological properties inside the cell and the nucleus and possibly associated with the fat-content. Consequently, chromosome breaks and gene changes depend to a less degree on the number of direct hits than on some physiological conditions which make the chromosomes break or change after the X-ray bombardment. Irradiation injuries imply secondary rather than immediate effects.

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DER ZUSAMMENHANG ZWISCHEN PERENNITÄT, APOMIXIS UND POLYPLOIDIE

VON *FOLKE FAGERLIND*
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STATISTISCHE Untersuchungen haben gezeigt, dass das Vorkommen von Polyploiden gewöhnlicher bei perennierenden als bei annuellen Pflanzen ist (vgl. beispielsweise MÜNTZING, 1936; STEBBINS, 1938). STEBBINS meint, dies beruhe darauf, dass die ersteren eben grössere Möglichkeiten hätten, Polyploide zu bilden: »If a sterile hybrid is annual, it must form in a single season either a tetraploid shoot or unreduced gametes which function. On the other hand a sterile perennial, if it is vigorous, may live indefinitely and even propagate itself vegetatively to form a large clone, as is illustrated by the classical examples of *Primula kewensis* and *Spartina Townsendii*, until finally the fortuitous combination of circumstances appears to produce the fertile amphidiploid». Der Gedankengang ist durchaus logisch. Die entgegengesetzte Ansicht, dass die Polyploidbildung primär wäre und die Perennität als Folge nach sich zöge, ist schon früher geäussert worden (LINDSTROM, 1936; MÜNTZING, 1936). Möglicherweise ist diese Ansicht zutreffend in einigen speziellen Fällen. Dass jedoch STEBBINS' Auffassung mehr generelle Gültigkeit besitzt, wird durch das Resultat einer Untersuchung an relativ engen systematischen Einheiten bewiesen, welchem Resultat natürlich grössere Beweiskraft zukommt als dem einer Untersuchung an grösseren Gruppen.

Die *Galium*-Gattung, bei der der Polyploidieeinschlag gross ist, zerfällt in mehrere wohlgeschiedene Sektionen, die kaum als polyphyletisch betrachtet werden können. Eine Prüfung ergibt hier folgendes Resultat (siehe Tabelle S. 180; vgl. FAGERLIND, 1937).

Die Sektionen innerhalb der Gattung *Galium* bestehen in der Regel nur aus Perennierenden (4 Sektionen) oder nur aus Annuellen (2 Sektionen). Für die Einteilung sind die Eigenschaften perennierend—annuell nicht massgebend gewesen. Hier liegt also kein Grund für die Annahme vor, dass der perennierende Typ durch das Auftreten von Polyploidie aus einem annuellen hervorgegangen sei. Die annuelle Gruppe *Aparine* enthält Fälle von hohem Polyploiditätsgrad. So kann

die reduzierte Zahl von 10 oder 11 bis auf mehr als 40 steigen, ohne dass die Pflanze deshalb weniger annuell wird. STEBBINS gibt an, dass in mehreren der in MÜNTZINGS (1936) Tabellen aufgeführten Gattungen die Verhältnisse die gleichen sind. Unter diesen kommt nur ein Fall vor, wo eine grössere Anzahl Arten untersucht worden und demungeachtet kein diploider Typ unter den perennierenden vorhanden ist — *Senecio*. STEBBINS gibt an, dass rein perennierende Gattungen einen grösseren Polyploidieeinschlag haben als rein annuelle Gattungen. Diese Übereinstimmung tritt jedoch nicht innerhalb der *Galium*-Sektionen zutage.

Galium-Sektion	Artanzahl mit dem Polyploiditätsgrad					Lebensform
	2	4	6	8	10	
Eugalium	14	11	4	1		Nur Perennierende
Leptogalium	1	3	1			Nur Perennierende
Trachygalium	3	1	1	1		Nur Perennierende
Platygalium		1	3		1	Nur Perennierende
Cruciata	1	1				Perennierende
	2					Annuelle
Aparine	4	5	2	1		Nur Annuelle
Pseudovaillantia	1	1				Nur Annuelle

Aufschlussreicher als ein Vergleich innerhalb Gattungssektionen sind Vergleiche zwischen den Mitgliedern einer intraspezifischen Polyploidiekette. Aus MÜNTZINGS Tabellen (1936, S. 343—350) und meiner Arbeit 1937 sind folgende Ketten genommen (siehe S. 182—183).

Aus der Liste, die leicht erweitert werden könnte, ist zu ersehen, dass viele polyploide Perennierende aus Perennierenden niedrigeren Polyploiditätsgrades hervorgegangen sind, und dass Annuelle bei Eintritt von Polyploidie ihre Lebensform behalten. Keine von den Ketten zeigt, dass die Lebensform wechselt, wenn der Polyploiditätsgrad steigt. Die Liste zeigt weiter, dass Serien von intraspezifischen Polyploiden gewöhnlicher unter Perennierenden als unter Annuellen sind. Dies kann natürlich teilweise darauf beruhen, dass die Perennierenden innerhalb der aufgeführten Gattungen gewöhnlicher gewesen sind als die Annuellen (vgl. *Galium*), oder darauf, dass die Perennierenden Gegenstand eingehenderer Studien gewesen sind. Doch kann dies nicht gut die eigentliche Ursache sein. Hier liegt vielmehr eine Erscheinung vor, die eine Stütze für STEBBINS' Ansicht abgibt.

Die Ketten von intraspezifischer Polyploidie zeigen, dass die grössere Fähigkeit der perennierenden Pflanze, Polyploide zu erzeugen, nicht auf die Fälle beschränkt sein kann, wo Bastardierung vorgelegen hat, welchen Fall STEBBINS zunächst im Auge hat. Folgendes ist natürlich ganz klar. Hat ein bestimmtes Individuum, mag es nun ein Bastard oder eine reine Art sein, eine gewisse, in den meisten Fällen freilich geringe Möglichkeit, unreduzierte Gameten hervorzubringen, so steigen die Chancen dafür, dass eine Kopulation zwischen solchen Gameten oder einem solchen und einem normalen Gameten stattfindet, und dass das Verschmelzungsprodukt einmal vital ausfällt, wenn die Anzahl Blüten und demnach die Samenanlagen- und Pollenmenge zunimmt. Hat ein solcher Typ nur wenige Blüten und ist er einjährig, ist er also im Jahre darauf nicht mehr vorhanden, so hat er in den meisten Fällen keine polyploide Nachkommen produzieren können. Ist der Typ dagegen perennierend, so bildet er Jahr für Jahr Blüten und kann dann vielleicht einmal einen vitalen polyploiden Typ herausbilden. Diese Überlegung ist hierin mit der STEBBINS' ziemlich identisch.

Infolge von meiotischen Störungen und oft auch anderswie verursachter Sterilität (vgl. MÜNTZING, 1936; FAGERLIND, 1937 und dort angeführte Literatur) besitzt der neugebildete polyploide Typ eine geringere Fähigkeit, sich auf sexuellem Wege zu reproduzieren, als ein diploider.

In der Nachkommenschaft der neugebildeten Polyploiden entsteht vielleicht allmählich ein Biotyp mit einer Genkombination, die Fertilität mit sich bringt (vgl. MÜNTZING, 1939). Handelt es sich um eine annuelle Form, so ist geringe Aussicht vorhanden, dass dieser fertile Biotyp sich herausbilden wird. Bei einer perennierenden Form sind die Chancen um ein Vielfaches grösser.

Dies würde erklären, dass in der Natur angetroffene Polyploide — inter- wie auch intraspezifische — zumeist vollwertige oder fast vollwertige Fertilität besitzen. Das nachstehend Angeführte deutet jedoch darauf, dass auch ein anderer Regulationsprozess wirksam ist.

WETTSTEIN (1937) hat gezeigt, dass ein neugebildetes polyploides *Bryum*-Individuum stark steril ist, sowohl was die Gameten als was die Sporen betrifft. Es ist ein ausgesprochener Gigastyp. Im Laufe der Zeit können indessen die Gigascharaktere allmählich abnehmen, und Hand in Hand damit geht eine Zunahme der Fertilität. Schliesslich (nach 11 Jahren) erhält man dann Produkte von »normaler» Grösse und Fertilität. Der intraspezifisch polyploide Typ hat sich zu einer neuen Art stabilisiert — in WETTSTEINS Versuch hatte sich *Bryum*

Art	Vorkommende Chromosomenzahlen									Lebensform		
Crepis gracilis	22	—	—	55	—	—	—	—	—	Beide	sind	annuell
» *acuminata*	—	33	44	55	—	—	—	—	—	Alle	»	perennierend
» *occidentalis*	22	—	44	—	—	—	—	—	—	»	»	»
Crucianella angustifolia	22	44	—	—	—	—	—	—	—	Beide	»	annuell
Asperula cynanchica coll.	22	44	—	—	—	—	—	—	—	»	»	perennierend
» *glauca*	22	44	—	—	—	—	—	—	—	»	»	»
Galium Mollugo coll.	22	—	44	—	66	—	—	—	—	Alle	»	»
» » *elatum*	22	—	44	—	—	—	—	—	—	Beide	»	»
» » *tirolense*	22	—	44	—	—	—	—	—	—	»	»	»
» *laevigatum*	—	—	44	—	—	—	88	—	—	»	»	»
» *verum*	22	—	44	—	—	—	—	—	—	»	»	»
» » *ruthenicum*	22	—	44	—	—	—	—	—	—	»	»	»
» *lucidum*	22	—	44	—	—	—	—	—	—	»	»	»
» *flavicans*	22	—	44	—	—	—	—	—	—	»	»	»
» *palustre*	22	—	—	—	66	—	88	—	—	Alle	»	»
» *uliginosum*	22	—	44	—	—	—	—	—	—	Beide	»	»
» *boreale*	—	—	44	—	66	—	—	—	—	»	»	»
» *rubioides*	—	—	—	—	66	—	—	—	132	»	»	»
» *aparine* (+ *Vaillantii*)..	22	—	44	—	66	—	88	—	—	Alle	»	annuell
» *spurium*	22	—	44	—	—	—	—	—	—	Beide	»	»
» *Parisiense*	22	—	44	—	66	—	—	—	—	Alle	»	»
» *setaceum*	22	—	44	—	—	—	—	—	—	Beide	»	»
Ranunculus acer	14	—	28	—	—	—	—	—	—	»	»	perennierend
» *auricomus*	—	—	32	—	48	—	—	—	—	»	»	»
» *lanuginosus*	14	—	28	—	—	—	—	—	—	»	»	»
» *repens*	16	—	32	—	—	—	—	—	—	»	»	»
Adonis vernalis	16	24	—	—	—	—	—	—	—	»	»	»
Papaver nudicaule	14	—	—	—	—	—	—	—	70	»	»	»

» *repens*	16	—	32	—	—	—	—	—	—	»	»	»
Adonis vernalis	16	24	—	—	—	—	—	—	—	»	»	»
Papaver nudicaule	14	—	—	—	—	—	—	—	70	»	»	»
Cardamine pratensis	—	—	32	—	—	—	64	—	—	»	»	»
Viola Kitaibeliana	14	—	—	36	—	—	—	48	—	Alle	»	annuell
Rumex domesticus	—	—	40	—	60	—	80	—	—	»	»	perennierend
Silene ciliata	24	—	48	—	—	—	—	—	192	»	»	»
» *vallesia*	24	—	48	—	—	—	—	—	—	Beide	»	»
Stellaria neglecta	22	—	44	—	—	—	—	—	—	»	»	annuell
Vicia cracca	12—14	—	28	—	—	—	—	—	—	»	»	perennierend
» *unijuga*	12	—	24	—	36	—	—	—	—	Alle	»	»
Medicago lupulina	16	—	32	—	—	—	—	—	—	Beide	bienn oder sub-perennierend	
Phleum alpinum	14	—	28	—	—	—	—	—	—	Beide	sind	perennierend
Phalaris arundinacea	14	—	28	—	—	—	—	—	—	»	»	»
Hordeum murinum	14	—	28	—	—	—	—	—	—	»	»	annuell
» *nodosum*	14	—	—	—	42	—	—	—	—	»	»	perennierend
» *secalinum*	14	—	28	—	—	—	—	—	—	»	»	»
Festuca myuros	14	—	—	—	42	—	—	—	—	»	»	annuell
» *arundinacea*	—	—	28	—	42	—	—	—	70	Alle	»	perennierend
» *ovina*	14	—	28	—	42	—	—	—	70	»	»	»
» *pratensis*	14	—	28	—	—	—	—	—	—	Beide	»	»
» *rubra*	14	—	—	—	42	—	56	—	70	Alle	»	»
» *varia*	14	—	28	—	42	—	—	—	—	»	»	»
Bromus inermis	—	—	—	—	42	—	56	—	—	Beide	»	»
Poa angustifolia	—	—	28	—	—	—	56	—	70	Alle	»	»
» *compressa*	—	—	—	—	42	—	56	—	—	Beide	»	»
» *nemoralis*	—	—	28	—	42	—	—	—	—	»	»	»
» *palustris*	—	—	28	—	42	—	—	—	—	»	»	»
» *pratensis*						50—84				Alle	»	»

Correnſii herausgebildet. Mit *Bryum Correnſii* übereinstimmende Formen sind später in der Natur angetroffen worden. Die Regressionsvorgänge sind also von evolutionärer Bedeutung.

Dieselben Differenzen, die zwischen neugebildeter, steriler und älterer, fertiler polyploider Form von *Bryum* bestanden, sind für die höheren Pflanzen bekannt. Beispiele von solchen Fällen wurden von FAGERLIND (1937) angeführt. Weitere Beispiele bieten durch Colchicineinwirkung neugebildete Polyploide von *Nasturtium officinale* und *Arenaria serpyllifolia*, die von HOWARD und MANTON (1940) bzw. WOESS (1941) erhalten worden sind. Diese beiden Polyploiden waren mehr gigasbetont, sowohl was die äussere Morphologie als was die Zellgrösse betrifft, und ferner zeigten sie geringere Fertilität als in der Natur bereits vorkommende Individuen desselben Polyploiditätsgrades.

Genau das Gleiche wie das, was WETTSTEINS Versuche zeigen, habe ich schon früher bei *Galium* und nahestehenden Gattungen zu sehen geglaubt (FAGERLIND, 1937).

Ich versuchte damals, die Veränderungen mit einer beobachteten Veränderung der Chromosomengrösse in Zusammenhang zu bringen und stellte eine Hypothese auf, deren Wahrheitsgehalt zu beurteilen vorläufig noch nicht möglich ist. Später hat man jedoch beobachtet, dass auch neugebildete Polyploide verkleinerte Chromosomen haben können (z. B. bei *Petunia*; LEVAN, 1939). WETTSTEIN und STRAUB (1942) haben angegeben, dass die Chromosomen selbst bei *Bryum Correnſii* nicht verkleinert sind. Es ist indes sehr eigentümlich, dass die Abbildungen, womit sie es demonstrieren wollen, Zellen zeigen, die viel grösser als abgebildete Zellen des diploiden Ursprungs sind. Ist eine Verwechselung ausgeschlossen? Die ebenerwähnten, neu synthetisierten *Arenaria 4-serpyllifolia* (4- bezeichnet einen intraspezifisch tetraploiden Typ; vgl. FAGERLIND, 1937) und *Nasturtium 4-officinale* haben deutlich grössere Chromosomen als in der Natur vorkommende tetraploide Parallelen. Die letzteren haben kleinere Chromosomen als die diploiden Parallelarten.

Sind derartige Regulationsprozesse, wie sie *Bryum Correnſii* geschaffen haben, generell wirksam, so ist es klar, dass Perennität grössere Möglichkeiten als Annuellität dafür bietet, dass ein steriler oder partiell fertiler Organismus vollwertig fertil wird. Wie dem nun auch sei, so ist jedenfalls auf Grund des Obigen die Schlussfolgerung berechtigt: *Der perennierende Typ hat im Vergleich zum annuellen eine grössere Möglichkeit, Polyploide zu bilden, und eine grössere Möglichkeit, ent-*

standene Polyploide zu bewahren. Letzteres spielt vermutlich die grössere Rolle für die Entstehung der behandelten Korrelation.

Ist da die Ansicht, dass Polyploidie Perennität bewirke, als hinfällig zu betrachten? Für einige Spezialfälle besitzt diese Ansicht wohl Gültigkeit, wofür Folgendes spricht.

1) *Oenothera Lamarckiana gigas* ist nach DE VRIES (1906) und GATES (1913, 1915) durch eine stärker ausgeprägte Tendenz zu Biennität als die »Ursprungstypen» gekennzeichnet. 2) *Nasturtium 4-officinale* ist perennierend, während der Ursprungstyp eine Tendenz zu Annuellität hat (MANTON, 1935). 3) SHARP (1934) gibt an, dass nach RANDOLPH (1932) *Zea 4-Mays*, von letzterem durch Wärmebehandlung von *2-Mays* erhalten, perennierend im Gegensatz zu dem annuellen Ursprungstyp sei. Man sucht indessen vergebens die Angabe in der angeführten Arbeit RANDOLPHS. 1931 veröffentlichte er jedoch eine Abhandlung, betitelt: »X-rayed seed of annual plant produces perennial». Diese Arbeit ist mir leider nicht zugänglich gewesen. Sie wird bei COLLINS und LONGLEY (1935) folgendermassen referiert: »From X-rayed material of annual teosinte he obtained a tetraploid that is practically indistinguishable from perennial teosinte». Offenbar bezieht sich SHARP auf diese Arbeit. Er hat *Zea* mit *Euclaena* verwechselt. 4) TISCHLER (1942) zitiert GLOTOV (1939) und sagt, dass *Mentha 4-piperita* durch Colchicinbehandlung von *2-piperita* erhalten, winterhart und perennierend ist. *Mentha 2-piperita* ist nicht winterhart. GLOTOV schrieb aber nur, dass Parallelversuche gezeigt haben, dass *4-piperita* im Gegensatz zu *2-piperita* ganz winterhart ist.

Eine hapaxantische Pflanze mit langer Vegetationsperiode kann zwei- oder mehrjährig sein. A priori anzunehmen, dass eine hapaxantische Pflanze durch Steigerung der Chromosomenzahl pollakantisch wird, liegt kein Grund vor. Dagegen lässt es sich sehr wohl denken, dass eine hapaxantische annuelle Pflanze durch die Polyploidie eine längere Vegetationsperiode erhält, zwei- oder mehrjährig wird. Dass der Eintritt von Polyploidie eine Verlängerung der »Vegetationsperiode» mit sich bringt, ist zuvor bekannt (vgl. MÜNTZING, 1936; FAGERLIND, 1937 und dort angeführte Literatur). Dies ist es wohl, was bei *Oenothera* geschehen ist, wo ja überdies schon der 2-Typ eine Tendenz zu Zweijährigkeit hat.

Ist eine Pflanze pollakantisch, aber infolge Mangels an Winterbeständigkeit annuell, so ist es möglich, dass sie bei eintretender Polyploidie perennierend werden kann. Mehrere Fälle von Polyploiden, die winterbeständiger sind als nahestehende Diploide, sind zuvor bekannt

(vgl. MÜNTZING, 1936; FAGERLIND, 1937 und dort angeführte Literatur). Dass die Perennität bei durch Colchicinbehandlung erhaltener *Mentha 4-piperita* (GLOTOV, 1939) auf die oben angeführte Weise erklärt werden kann, ist sicher. Ob der Fall bei dem erwähnten Teosintegras auch so erklärt werden kann, ist mir unbekannt. Da das perennierende und polyploide Produkt durch Behandlung mit Röntgenstrahlen erhalten wurde, ist es ebenso wahrscheinlich, dass der Effekt durch Mutation zustande kam.

Mehrere Fälle von Perennität, bedingt durch Innovationssprosse verschiedener Art, können ebensogut als Fälle von Apomixis betrachtet werden. In diesen Fällen ist es den vorstehenden Darlegungen gemäss wahrscheinlich, dass die Polyploidie sekundär und die Perennität oder die Apomixis das Primäre ist. Wie liegt die Sache nun in anderen Fällen von Apomixis?

Schon früh ist nachgewiesen worden, dass die agamospermischen Pflanzen, verglichen mit verwandten Gamospermen, einen höheren Polyploiditätsgrad besassen (vgl. Zusammenstellungen bei ROSENBERG, 1930 und GUSTAFSSON, 1935). Bisher sind unter den agamogonischen Pflanzen *Alnus rugosa* (WOODWORTH, 1930), *Potentilla* spp. (MÜNTZING, 1928, 1931; POPOFF, 1935; RUTISHAUSER, 1943) und *Hieracium umbellatum* (GENTSCHEFF, 1941) die einzigen bekannten diploiden Typen.

Hierher rechne ich nicht die diploiden *Trillium grandiflorum, erectum, undulatum* und *sessile*, trotz der von JEFFREY und HAERTL (1939—40) gelieferten Mitteilung. Diese Verfasser behaupten, dass hier reduzierte, monosporische, vierkernige Embryosäcke vorkommen, die parthenogenetisch haploide Embryonen und diploides Endosperm produzieren. Das letztere soll seinerseits direkt diploide Embryonen erzeugen. Da sie keinerlei Tatsachen zur Stütze ihrer Ansicht beibringen, ist diese meines Erachtens als unzutreffend zu betrachten.

Aposporie oder eine andere Form von apomeiotischer Embryosackbildung, die als ein Vorstadium zu Agamospermie betrachtet werden kann, ist jedoch bei einigen anderen Diploiden bekannt, bei *Oxyria* (EDMAN, 1929), *Picris* und *Leontodon* (BERGMAN, 1935) und Farnen (ANDERSSON-KOTTÖ, 1932).

Den agamogonischen Pflanzen können unter den Tieren Typen mit thelytokischer Parthenogenese gleichgestellt werden. Unter diesen finden sich sicher diploide Typen. *Rhabditis »XIX»* (BELAR, 1923, 1928) hat sogar eine sehr niedrige Chromosomenzahl. Parthenogenetische *Heterocypris incongruens* weist denselben Polyploiditätsgrad auf wie

die sexuelle (BAUER, 1940). *Polydrosus mollis* ist eine diploide Apomikte, während die Apomikten in den Gattungen *Otiorrhynchus*, *Strophosomus*, *Trachyphloeus*, *Sciaphilus* und *Barynotus* tri- oder tetraploid sind (SUOMALAINEN, 1940 a und b). Nahestehende Sexuelle waren durchgehends diploid. In anderen Gruppen sind die Apomikten intraspezifische Polyploide verschiedenen Grades. Hierher gehören Vertreter der Gattungen *Solenobia* (SEILER, 1923, 1927), *Trichoniscus* (VANDEL, 1927 a und b), *Cypris* (SCHLEIP, 1909), *Artemia* (ARTOM, 1928; GROSS, 1935; BARRIGOZZI, 1934 a und b, 1936 a und b), *Carausius* (PEHANI, 1925; CAPPE DE BAILLON, 1931; CAPPE DE BAILLON, FAVRELLE und DE VICHET, 1935), *Daphnia* (?) (SCHRADER, 1925). In allen diesen Fällen mit Ausnahme von *Artemia* und *Solenobia* bestehen die entsprechenden Diploiden nur aus sexuellen Individuen. Von *Artemia* gibt es auch apomiktische Diploide. Die Tiere mit thelytokischer Parthenogenese weisen also dieselben Verhältnisse auf wie die agamogonischen Pflanzen.

Auch Pflanzen mit Nuzellarembryonie (extrasakkaler Embryonie) sind meistens Polyploide. Die einzigen Ausnahmen, die ich kenne, sind *Euphorbia dulcis* (CARANO, 1926), sowie Mitglieder der Gattungen *Citrus*, *Poncirus*, *Fortunella* (FROST, 1925, 1926, 1938 b) und *Eugenia* (VAN DER PIJL, 1934).

Fortpflanzung mittels Bulbillen und Brutknospen kommt bei einigen Pflanzen vor und oft da verbunden mit m. o. w. starker Abschwächung der gamospermischen Vermehrung. Diese Pflanzen sind oft durch höheren Polyploiditätsgrad charakterisiert als nahe Verwandte mit normaler Reproduktion. So ist der, praktisch genommen, ganz apomiktische *Ranunculus ficaria* tetraploid, der sexuelle Typ diploid (MARSDEN-JONES, 1935). Nach TURESSON (1930, 1931) sind die polyploiden *Festuca ovina*-Biotypen vivipar, die diploiden gamospermisch. *Dentaria bulbifera* ist tetraploid. Alle nahen Verwandten dieser Art sind diploid und haben keine Brutzwiebeln (SCHWARZENBACH, 1922). *Polygonum viviparum* hat eine höhere Chromosomenzahl als die meisten *Polygonum*-Arten (JARETZKY, 1928). Weitere ähnliche Beispiele liessen sich sicher noch beibringen. Innerhalb dieser Klasse ist jedoch die apomiktische Fortpflanzung nicht immer auf die polyploiden Typen beschränkt. Grasarten sind bekannt, die vivipar sind und keine höhere Chromosomenzahl haben als nahverwandte amphimiktische Arten (STÄHLIN, 1929; vgl. auch TURESSON, 1930). Bei den *Allium*-Arten von der Sektion *Paniculatum* gibt es Diploide mit, Diploide ohne und Polyploide mit Brutknospen (LEVAN, 1937).

Pflanzen, die sich auf andere Weise vegetativ vermehren, zeigen ebenfalls oft einen höheren Polyploiditätsgrad als nahe Verwandte, die dieser Fähigkeit ermangeln. Der 4-Typ von *Biscutella laevigata* (MANTON, 1934) vermehrt sich durch Wurzelschösslinge. Solche kommen bei dem 2-Typ nicht vor. *Helianthus tuberosus* mit seinen unterirdischen Vermehrungssprossen hat eine bedeutend höhere Chromosomenzahl als *H. annuus*, der solcher Sprossen ermangelt (TISCHLERS Tabellen). Auch innerhalb dieser Klasse liessen sich sicher noch mehr Beispiele antreffen. Auch finden sich viele Fälle, wo eine Übereinstimmung nicht vorhanden ist, oder wo die Verhältnisse die gerade umgekehrten sind. Zu den letzteren gehört *Empetrum nigrum*, dessen diploider Typ in höherem Grade vegetativer Vermehrung fähig ist als der tetraploide (HAGERUP, 1927).

Die Forscher, die früher versucht haben, den Zusammenhang zwischen Polyploiditätsgrad und Apomixis zu erklären, haben alle vermutet, dass die Polyploidie irgendwie zur Entstehung der letzteren beigetragen hat. Viele haben auch die Apomikten als Artbastarde betrachtet. 1941 entwickelt STEBBINS hiermit übereinstimmende Ansichten. Bei *Crepis*, wo die Agamospermen Polyploide, und zwar nach STEBBINS zumeist interspezifische Polyploide sind, denkt er sich das Zustandekommen derselben folgendermassen. Durch Kreuzung der sexuellen diploiden Arten sind sterile Bastarde entstanden. Durch diese Bastardierung werden komplementäre Gene zusammengeführt, die für die Realisierung der Agamospermie Bedeutung haben. Die sterilen Bastarde können dann durch Chromosomenverdoppelung fertile Typen bilden, unter denen oder unter deren Nachkommen apomiktische Formen vorkommen. Die Polyploidie ist, meint er, in den meisten Fällen notwendig für »the full expression of these genes and probably acts as modifying agent by changing the physiological condition of the cells». BABCOCK und STEBBINS (1938) nehmen an, dass in fast allen agamischen Komplexen fakultative Apomikten den obligaten vorausgegangen sind.

Dass die Apomixisphänomene und Aposporie tatsächlich genbedingt sind, zeigen Beobachtungen von OSTENFELD (1910), LIDFORSS (1914), MÜNTZING (1940; vgl. auch HÅKANSSON, 1943), ANDERSSON-KOTTÖ (1932), BERGMAN (1935) und CHRISTOFF (1942). Im Zusammenhang mit STEBBINS' Ansicht, dass die Apomixis in den meisten Fällen durch Zusammenwirkung zwischen komplementären Genen und Polyploidie hervorgerufen sei, ist es von Interesse, auf folgende Verhältnisse bei Mitgliedern der Gattungen *Allium* und *Festuca* hinzuweisen.

Allium oleraceum ist tetraploid und hat immer Brutknospen. Aus zwei diploiden *Allium paniculatum*-Typen ohne Brutknospen gelang es LEVAN (1937), *Allium oleraceum* zu synthetisieren. In derselben Kreuzung erhielt er fünfzehn diploide Pflanzen, die Brutknospen entbehrten. Bei *Festuca ovina* nimmt die amphimiktische Reproduktion, die neben der apomiktischen vorkommt, gradweise ab, um schliesslich bei hohem Polyploiditätsgrad ganz zu verschwinden (TURESSON, 1930). Unter den Gramineen ist es aber auch bekannt, worauf TURESSON (1930) hinweist, dass ausser der erblichen Viviparie auch modifikativ hervorgerufene Viviparie vorkommt. TINCKER (1925) und THOENES (1929) haben nämlich nachgewiesen, dass eine solche durch Änderung der Lichtverhältnisse und des Feuchtigkeitsgrades hervorgerufen werden kann. Da bekanntlich die osmotischen und physiologischen Eigenschaften einer Pflanze (vgl. MÜNTZING, 1936; FAGERLIND, 1937 und dort angeführte Literatur) sich mit eintretender Polyploidie ändern, liegt es ausserordentlich nahe, auf Grund der Verhältnisse bei den Gramineen eine Hypothese über den Zusammenhang zwischen Apomixis und Polyploidie aufzustellen. Durch den Eintritt der Polyploidie werden Verhältnisse geschaffen, die gleicher Art wie die sind, welche durch die Milieuvariationen hervorgerufen wurden, wenn eine diploide, sonst normale Pflanze unter den Gramineen vivipar wurde. Hier wird nun aber die Viviparie erblich (konstant). Liegen die Verhältnisse derart, so ist die Polyploidie eine mitwirkende Ursache der Entstehung der apomiktischen Reproduktion. Die Erklärung mag vielleicht die richtige für einige Fälle sein (*Festuca ovina*, *Allium oleraceum*), dass sie aber generell gilt, ist ausgeschlossen. Es gibt ja auch diploide Apomikten. Möglicherweise werden bei diesen durch ihre spezielle genetische Konstitution dieselben osmotischen und physiologischen Verhältnisse geschaffen wie bei den Polyploiden.

Wir wissen, dass Polyploidie nicht von Apomixis begleitet zu sein braucht — nicht einmal innerhalb der Gattungen *Taraxacum* und *Hieracium*, die starke Einschläge von Agamogonie aufweisen, werden synthetisierte intra- und interspezifische Polyploide zu Apomikten (KOSTOFF und TIBER, 1939; KOROLEVA, 1939; PODDUBNAJA-ARNOLDI, 1939; CHRISTOFF, 1940) — sowie dass es Apomikten gibt, die nicht polyploid sind. Oben ist auch gezeigt worden, dass die apomiktische Reproduktion in verschiedenen Fällen ganz verschiedene Wege eingeschlagen hat (Agamogonie, Nuzellarembryonie, Viviparie und viele andere Formen von Innovationssprossen), dass aber demungeachtet die Korrelation stets augenfällig ist. In allen diesen Fällen eine Kombina-

tion zwischen gewissen komplementären Genen und durch das Eintreten der Polyploidie veränderten physiologischen Verhältnissen anzunehmen, erscheint wenig glücklich. Ungesucht stellt sich hier die Frage ein, ob es nicht denkbar ist, dass die Apomixis das Primäre und die Polyploidie das Sekundäre sein kann. Zwei Möglichkeiten kommen da in Betracht, die zur Entstehung der Korrelation beitragen können, nämlich: 1) die Apomixis erhöht die Chancen für die Bildung von Polyploiden und 2) die Apomixis bewirkt die Erhaltung der entstandenen Polyploiden.

Stellen wir hier dieselbe Überlegung wie zur Beleuchtung des Zusammenhanges zwischen Perennität und Polyploidie an, so zeigt es sich, dass die beiden Möglichkeiten vorliegen müssen. Da die apomiktische Vermehrungsweise, verglichen mit Perennität, ein bedeutend effektiverer Weg ist, einen Biotyp am Leben zu erhalten, so müssen sie in verschärftem Grade vorliegen. Dass die Apomixiserscheinung den Fortbestand einer entstandenen Polyploiden begünstigt, liegt übrigens offen zutage. Mehrere Apomikten — die triploiden — könnten überhaupt nicht existieren, wenn sie sexuell wären.

Oben habe ich darauf hingewiesen, dass neugebildete Polyploide offenbar erst dann fertil werden, wenn sie durch Genrekombination oder auf andere Weise »umgeändert» worden sind. Kommt es nicht zu dieser Umwandlung, so stirbt die Polyploide aus. Bei einer Apomikte ist diese »Umwandlung» unnötig. Die Erhaltung entstandener Polyploiden durch das Apomixisphänomen ist vermutlich von grösster Bedeutung für die Entstehung der hier fraglichen Korrelation.

Mehrere Apomikten haben gestörte Gametophytogenese, so dass unreduzierte Gameten in verhältnismässig hoher Frequenz vorliegen. Dass die Eier in den unreduzierten Embryosäcken der Agamogonen befruchtungsfähig sind, wissen wir (*Hypericum*; NOACK, 1939. *Rubus*; PETROV, 1939. *Potentilla*; MÜNTZING und MÜNTZING, 1942). Eine ganz andere Möglichkeit, Polyploide zu bilden, haben auch die Apomikten, wenn somatische Verdoppelung eben in den Geweben geschieht, in welchen die Initialzellen für die nächste Generation gebildet werden. Die hier angestellte Überlegung steht in Übereinstimmung mit der Auffassung, dass das nachgewiesene regelmässige Auftreten von Tetraploiden in der Nachkommenschaft von diploidem *Citrus limonum* durch Nuzellarembryonie und »Syndiploidie» im Nuzellusgewebe bedingt ist (FROST, 1925).

Eine Stütze für den Gedanken, dass, wenn Apomixis und Polyploidie zusammen auftreten, die erstere das Primäre und die letztere das Sekundäre sein kann, erblicke ich in der Tatsache, dass innerhalb

gewisser grösserer oder kleinerer Gruppen von Organismen anscheinend die Polyploidie ausschliesslich an die apomiktischen Typen gebunden ist. Die Gattung *Elatostema* gehört vielleicht hierher (FAGERLIND, 1943). Das schönste Beispiel innerhalb des Pflanzenreichs ist jedoch *Crepis*. Hier kommt Polyploidie innerhalb der Sektion, welcher die Agamogonen angehören, nur bei den apomiktischen Formen vor. Hier springt auch die Polyploidiekette hoch in die Höhe. Polyploidie ist in dieser Sektion in der Natur ausserordentlich viel gewöhnlicher als in den übrigen Sektionen, in welchen Agamospermie nicht vorkommt. Analoge, sehr instruktive Fälle bilden die Tiere mit thelytokischer Parthenogenese. Im Tierreich sind ja Polyploide und polyploide Serien sehr selten. Intraspezifische Polyploidie ist nur gewöhnlich im Zusammenhang mit Apomixis. Im letzteren Falle kann die Chromosomenzahl stark emporsteigen. Die Basis der genannten Polyploidieketten bilden Diploide, unter denen nur in Ausnahmefällen Apomikten bekannt sind. Schon der nächste Schritt in der Kette, die tri- oder tetraploiden Typen, wird nur durch die Apomikten repräsentiert. Sodann folgen die übrigen Glieder der Kette mit höherem Polyploiditätsgrad. Auch diese sind Apomikten. Die höherploiden Apomikten müssen demnach von den tri- oder tetraploiden Apomikten gebildet worden sein, die also eine grössere Fähigkeit besitzen, vitale Polyploide zu liefern, als die sexuellen Formen. Die bei *Crepis* und gewissen Tieren vorliegende ungewöhnliche Höhe der Kette muss somit durch die Apomixis bedingt sein.

Ist nun die Apomixis durch Gene bedingt, und sind die primären apomiktischen Polyploiden oft intraspezifische Polyploide, und ist ferner die Apomixis eine primäre, die Polyploidie eine sekundäre Erscheinung, so müssen ausser den polyploiden Apomikten derselben Art angehörige diploide Apomikten existieren oder früher existiert haben. Derartige Apomikten aus dem Tierreich sind bereits oben erwähnt worden. Hierher gehören aus der Pflanzenwelt *Potentilla*-Arten (MÜNTZING, 1928, 1931) und *Hieracium umbellatum* (ROSENBERG, 1917; BERGMAN, 1935 b; GENTSCHEFF, 1941). Mehreren anderen Apomikten entsprechen zwar Diploide, aber diese sind nicht Apomikten. Von solchen sind beispielsweise zu nennen *Chara crinita* (ERNST, 1918) — falls nun die apomiktischen Formen von *Chara crinita* als solche mit Chromosomenverdoppelung aufzufassen sind [1] —, *Nigritella nigra* (AFZELIUS, 1928),

[1] Dass, wenn Agamogonie bei den Angiospermen vorliegt, die Apomeiosis das Primäre ist, geht daraus hervor, dass haploide agamogone Pflanzen unbekannt sind, und dass partiell agamogone Pflanzen nicht in grösserem Umfang Nachkommen mit reduzierter Chromosomenzahl bilden. Wenn die Verhältnisse bei *Chara crinita*

Crepis spp. (BABCOCK und STEBBINS, 1938; STEBBINS und JENKINS, 1939), *Ranunculus ficaria* (MARSDEN-JONES, 1935), *Trichoniscus* (VANDEL, 1927 a und b), *Solenobia* (SEILER, 1923, 1927) und *Daphnia* (?) (SCHRADER, 1925).

Von den von SUOMALAINEN (1940 a) beschriebenen tri- und tetraploiden Curculionidae kennen wir zwar nicht sicher diploide, sexuelle Entsprechungen. Man darf es jedoch trotzdem als ziemlich sicher ansehen, dass solche existieren. Sämtliche sexuelle Arten, die er untersucht hat, waren diploid. APFELBECK (1928—29) schreibt von der Gattung *Otiorrhynchus*, die zu den von SUOMALAINEN untersuchten gehört: »Zu jeder parthenogenetischen Art existiert nach meiner bisherigen Erfahrung stets eine mehr minder nahverwandte — oft täuschend ähnliche — zweigeschlechtliche Art, die sich aber im Vorkommen gegenüber der andern stets ausschliesst, d. h. es kann die eine oder andere Art — wie z. B. *O. niveopictus* und *sarajevensis* — wohl in demselben Gebirge vorkommen, niemals aber an derselben oder einer benachbarten Lokalität». APFELBECK zählt 18 solche Fälle mit Parallelarten auf. Aus seinen Beschreibungen geht hervor, dass die Differenz zwischen den Parallelarten oft hauptsächlich quantitativen Charakters ist. Unter den 18 Arten befinden sich eben zwei der von SUOMALAINEN untersuchten Apomikten: *O. ovatus* und *O. ligustici*. Die erstere Art hat nach APFELBECK eine Verbreitung über Europa bis nach Albanien, die letztere kommt in Europa und Kleinasien vor. Die erstere hat in Siebenbürgen eine sexuelle Parallelform in *O. Schusteri*, die letztere in Kaukasien, Südrussland, Thessalien und Bulgarien eine sexuelle Parallele in *O. subrotundatus*. Alles spricht also dafür, dass die tri- und tetraploiden apomiktischen Curculionidae an gewissen Lokalitäten diploide, sexuelle Parallelen haben. Natürlich ist es nicht ausgeschlossen, dass auch apomiktische, diploide Parallelen existieren.

Dass vielen apomiktischen Polyploiden nicht apomiktische oder

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gleichartig sind, erhält man ohne weiteres die Erklärung dafür, dass die apomiktischen Typen eine doppelt so grosse Chromosomenzahl wie die amphimiktischen haben, wie dies von ERNST (1918) nachgewiesen worden ist. Bei *Chara* fehlt ja eine Sporophytengeneration, oder, anders ausgedrückt, die Sporophytengeneration ist nur durch eine einzige Zelle, die Zygote, repräsentiert, bevor die Reduktionsteilung eintritt. Wenn hier Apomeiosis vorliegt, wird ja die hervorsprossende Pflanze diploid, nicht haploid. Bei den Angiospermen gelangt man nicht in ebenso einfacher Weise zu einer Erklärung. Vergleicht man hier die Gametophyten einer apomiktischen Pflanze, die tetraploid ist, mit den Gametophyten einer diploiden amphimiktischen Form, so ist das Verhältnis zwischen den Chromosomenzahlen nicht 2 : 1, wie bei *Chara crinita*, sondern 4 : 1.

nicht einmal sexuelle Diploide entsprechen, kann auf einer reinen Konkurrenzerscheinung beruhen. Ist eine Entsprechung vorhanden, so macht diese oft den Eindruck, von Reliktcharakter zu sein. Sie ist auf ein kleines Verbreitungsgebiet beschränkt und weist oft deutliche Zeichen auf, dass sie in Rückgang begriffen ist, während die apomiktische Polyploide einen vitaleren Eindruck macht und ein relativ grösseres Verbreitungsgebiet hat, das bisweilen ein ganz anderes ist. Extreme Beispiele hierfür sind *Chara crinita* und *Solenobia*. Die erstere Art ist offenbar im Aussterben begriffen. Auf ihren wenigen Standorten gewinnen die Apomikten mehr und mehr die Oberhand. Die männlichen Individuen sind zu bekannten Zeiten an den ältesten bekannten Lokalitäten ausgestorben. Gleichzeitig müssen natürlich die nichtapomiktischen weiblichen Individuen an derselben Lokalität ausgestorben sein. Die diploide sexuelle *Solenobia* ist nur an Lokalitäten um Nürnberg und Zürich angetroffen worden (SEILER, 1927).

Gleichartig, aber nicht so extrem ist — um ein Beispiel aus dem Pflanzenreich zu wählen — der *Crepis*-Fall. Die Agamogonen der Gattung haben eine Verbreitung über grosse Teile des nordamerikanischen Kontinents (vgl. BABCOCK und STEBBINS' Karten). Von den 7 nichtapomiktischen Parallelarten hat eine einzige grosse Verbreitung, die übrigen sind noch in den relativ kleinen Gebieten erhalten, die als die primären Heimatgebiete der Gattung betrachtet werden (vgl. BABCOCK und STEBBINS, 1938; STEBBINS, 1941). Gleicher Art sind auch mehrere obenerwähnte Fälle.

Wir wissen, dass die Polyploiden in der Natur oft andere und grössere Gebiete bevölkern als die entsprechenden Diploiden (vgl. FAGERLIND, 1937 und dort angeführte Literatur), sowie dass sie hinsichtlich der Konkurrenz begünstigt sind. In nicht wenigen Fällen haben die Polyploiden sehr grosse Verbreitung, während die diploiden Parallelformen nur innerhalb eines relativ kleinen Gebiets vorkommen. Als Beispiele hierfür führte TISCHLER (1942) an *Capsella bursa pastoris*, *Solanum nigrum*, *Fumaria officinalis*, *Erodium cicutarium* und *Rumex acetosella*. Wenn nun eine diploide Art teils aus sexuellen Formen — die grosse Masse — besteht und teils aus einer bedeutend geringeren Anzahl Apomikten und die ganze Masse der Diploiden auf ein kleineres Gebiet eingeengt oder gänzlich zum Verschwinden gebracht wird, während die mit Chromosomenverdoppelung ausgestatteten apomiktischen Derivate völlig neues Land erobern, so muss ja grosse Gefahr vorliegen, dass zunächst die diploiden Apomikten und dann auch die sexuellen Formen aussterben. Man hat daher wenig Möglichkeiten, sie anzutreffen.

Eine Bestätigung der Richtigkeit dieser Überlegung sehe ich in den Fällen *Artemia salina* und *Hieracium umbellatum*. Von beiden kennt man diploide, sexuelle Formen. Bis vor kurzem meinte man, dass die agamospermischen *Hieracium umbellatum*-Formen durchweg triploid und dass sie in Asien beheimatet wären. Neulich teilte jedoch GENTSCHEFF (1941) mit, dass er auch diploides, agamospermisches *Hieracium umbellatum* angetroffen habe. Auch von *Artemia salina* kennt man nun diploide Apomikten (GROSS, 1932, 1935; BARRIGOZZI, 1934, 1936). Vor diesen Funden GENTSCHEFFs bzw. GROSS' und BARRIGOZZIS erinnerten die Fälle *Artemia salina* und *Hieracium umbellatum* an den Fall *Chara crinita* und ähnliche, wo die polyploide Apomikte eine bestimmte geographische Verbreitung hat, eine diploide Apomikte unbekannt ist und eine sexuelle Diploide mit m. o. w. unbedeutender Ausbreitung vorkommt. Es ist daher mehr als wahrscheinlich, dass intensiver betriebene Untersuchungen früher oder später zur Entdeckung des Vorkommens apomiktischer Stämme unter den diploiden Parallelformen zu den polyploiden Apomikten führen werden.

Dass Apomixis wirklich der primäre Zustand sein kann, der sekundär die Bildung lebensfähiger Polyploiden ermöglicht hat, ist bereits von den Apomixisforschern, die sich mit zoologischen Objekten beschäftigt haben, eingesehen worden. Da *Solenobia triquetrella* auch durch diploide Apomikten vertreten ist, meint SEILER (1939), dass die polyploiden Apomikten der Gattung durch einen automiktischen Prozess entstanden seien. Dass derartige automiktische Prozesse, die zur Erhöhung des Polyploiditätsgrades bei apomiktischen Tieren führen, tatsächlich vorkommen können, wissen wir, da diese Erscheinung bei *Artemia salina* von GROSS (1932) beobachtet worden ist. Es ist dies von besonderem Interesse, da auch *Artemia salina* zu den Arten gehört, die ausser sexuellen und apomiktischen Polyploiden auch diploide apomiktische Individuen aufweisen. SUOMALAINEN ist auch davon überzeugt, dass Apomixis und Polyploidie bei den Tieren zu verschiedenen Zeitpunkten entstanden sind, und dass die Apomixis zuerst aufgetreten ist; er sagt ausdrücklich: »Die Parthenogenese macht bei Tieren die Polyploidie möglich».

Fassen wir unsere Erörterung zusammen, so können wir sagen, *dass es ebenso leicht oder sogar leichter ist, die Korrelation zwischen dem Vorkommen von Apomixis und Polyploidie zu verstehen, wenn man annimmt, dass die erstere Erscheinung primär, die letztere sekundär ist, als wenn man das Umgekehrte annimmt.* (Ausgeschlossen ist es natürlich nicht, dass in dem einen Falle die eine Hypothese, in einem

anderen die andere der Wirklichkeit entspricht.) *Der Kausalzusammenhang kann demnach der gleiche sein, wie er es nach* STEBBINS' *und meiner Ansicht meistens ist, wenn es sich um die Korrelation zwischen dem Vorkommen von Perennität und Polyploidie handelt. Dafür, dass in beiden Fällen ein gleicher Kausalzusammenhang vorliegt, spricht die Tatsache, dass Perennität und Apomixis die gleiche Folge haben, nämlich Verlängerung der Lebensdauer einer bestimmten Genkombination.*

Ist die Vorstellung von der Ursache dafür, dass die diploiden Parallelarten der polyploiden Apomikten nunmehr ganz fehlen oder geringe Verbreitung haben, richtig, so muss sie auch für andere Polyploide gelten. Die Schlussfolgerung wäre da die, dass eine ganze Reihe in der Natur vorhandener Polyploiden heute diploider Parallelen entbehren. Ein Teil dieser Polyploiden kann da, obwohl man Diploide, die derselben Art zugewiesen werden können, nicht kennt, ursprünglich intraspezifischen Charakters sein (vgl. MÜNTZING, 1936, 1940; FAGERLIND, 1937, 1943; TISCHLER, 1942).

ZUSAMMENFASSUNG.

1. Gewisse Tatsachen sprechen zugunsten der Hypothese, dass der Zusammenhang zwischen dem Vorkommen von Polyploidie und Perennität in den meisten Fällen auf der grösseren Fähigkeit der perennierenden Pflanzen beruht, Polyploide zu bilden und neugebildete Polyploide beizubehalten.

2. Die zuletzt von STEBBINS verfochtene Ansicht, dass der Zusammenhang zwischen dem Vorkommen von Polyploidie und apomiktischer Fortpflanzungsweise darauf beruht, dass letztere durch ein Zusammenspiel zwischen komplementären Genen und Polyploidie hervorgerufen wird, erhält Stützen durch einige Tatsachen.

3. Noch weitere Umstände werden angeführt, die die Annahme begründet erscheinen lassen, dass die in Punkt 2 erwähnte Korrelation in den meisten Fällen auf der grösseren Fähigkeit der Apomikten beruht, Polyploide zu bilden, und vor allem auf ihrer Fähigkeit, neugebildete Polyploide beizubehalten.

4. Dass den apomiktischen Polyploiden nur selten apomiktische diploide Parallelformen entsprechen, beruht, allem nach zu urteilen, auf Konkurrenzwirkung.

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CONTRIBUTIONS TO THE GENETICS AND CYTOLOGY OF MIRABILIS

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I. INTRODUCTION.

THE genus *Mirabilis*, especially the species *Jalapa* and *longiflora*, has been the subject of many experiments, but so far an F_2 analysis of the species cross has not been undertaken (for the older literature, cf. FOCKE, 1881, and CORRENS, 1902). The cytological study of *Mirabilis* presents many difficulties. TISCHLER (1908, 1928, 1929) finds 27 (or 28) as haploid chromosome number and SHOWALTER (1935) 29.

The genetical part of the present investigations was carried out at the Laboratory of Genetics in Wageningen in the years 1930—1939. From the cross *Mirabilis Jalapa* ♀ × *M. longiflora* ♂ ten fruits (or »seeds») and F_1 plants were obtained, while the reciprocal cross, just as in all earlier experiments, did not succeed. Most of the F_1 plants are still alive, thanks to the tuberously thickened roots, which have grown to mighty dimensions. The most fertile F_1 plant (No. 7) was used as mother plant for the F_2 generation. Up to 1939 more than one thousand F_2 plants were grown and studied in Wageningen. The cytological investigations were made at the Institute of Genetics in Lund in the years 1940—1942. Most of the fixations (root tips after cooling) were performed with diluted chrome-acetic-formalin, and the slides were stained with gentian violet.

II. GENETICAL ANALYSIS.

In the parent species, F_1 and F_2, the following characters were studied: growth habit, flower colour, length of flower, and hairiness of leaves. The original lists of descriptions and measures are not at my disposal. A preliminary analysis during the course of the investigations, however, showed that the F_2 segregation was essentially the same year after year. These preliminary results will be briefly communicated here. In a later paper I hope to give a more detailed description and analysis.

Growth habit. — In *Mirabilis Jalapa* the short stem is erect and the rather stout main branches are nearly so; the growth habit is bush-like (height and diameter ± 7 dm.). *M. longiflora*, on the contrary, has no vertical stem and the slender main branches are horizontal, pressed to the ground; the side branches are ascendent (plant height ± 4 dm., diameter ± 13 dm.). The growth habit of the F_1 resembles that of the mother, *M. Jalapa*. Its branches are, however, longer and more slender, and the plants show some hybrid vigour (height ± 8 dm., diameter ± 15 dm.). The F_2 generation is extremely multiform and a classification into growth types is very difficult. The growth habit in *Mirabilis* mainly depends upon the direction of stem and main branches, but many other characters contribute to it: length and number of the main branches; direction, length and number of the side branches; thickness of the nodes and thickness and length of the internodes; such characters as size, colour and hairiness of the leaves may also be of some importance. Taking into account the direction of the main branches only, a rather simple factorial analysis may be possible.

Flower colour. — In the *Mirabilis Jalapa* line used the flower colour is pale yellow and the whole plant is without anthocyanine colour. In *M. longiflora* the flower limb has an extremely faint lilac tinge and may be considered to be practically white; in the upper end of the flower tube it has a violet, anthocyanine coloured ring, and anthocyanine is found in the branches also. F_1 has a pale lilac flower and some anthocyanine in the branches. F_2 shows a clearly monofactorial segregation into plants with and without anthocyanine in flowers and branches. A small F_2 family grown in Sweden segregated into: 153 with anthocyanine and 48 without (expected 50,25), together 201, D/m = 0,37. The plants with anthocyanine in branches and flowers can be divided into two groups, those with lilac and those with pink flower colour. Segregation into lilac and pink is also clearly monofactorial: lilac 105 and pink 33 (expected 34,5), together 138, D/m=0,30. (Among all the investigated F_2 plants, more than one thousand, two showed a slightly deviating brownish lilac tinge, cf. next page).

The intensity of the lilac or pink flower colour in F_2 shows a very great variation, from practically white to rather deep lilac or pink, much darker than F_1. A sharp classification into classes appeared quite impossible. It seemed, however, as if a very faint colour class could be distinguished rather clearly from the rest. This class comprised every year about one sixteenth of the lilac and pink flowering plants. In this faint-colour group, however, only a smaller part of the

plants have flowers which are as »white» as the *longiflora* flowers. Therefore, at least three intensity factors, (I_1, I_2, I_3) must be supposed to be cryptomerically present in *M. Jalapa*. As *M. longiflora* has a dominant factor for anthocyanine (A) and for the lilac tinge (P) versus pink, the formulae for flower colour should be: *Mirabilis Jalapa* $aa\ pp\ I_1\ I_1\ I_2\ I_2\ I_3\ I_3$ and *M. longiflora* $AA\ PP\ i_1\ i_1\ i_2\ i_2\ i_3\ i_3$.

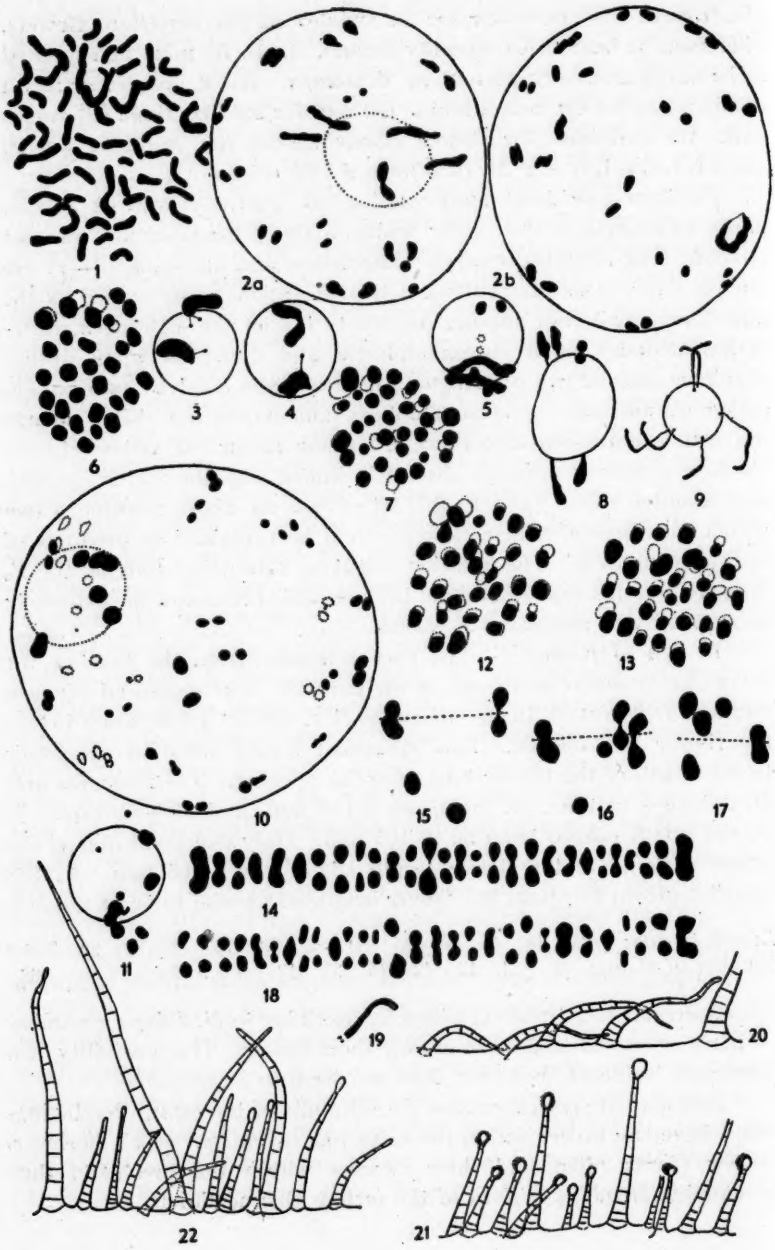
F_2 flowers without anthocyanine are partly yellow as *Jalapa*, partly paler yellow and partly white; a sharp classification was not possible. The relations between white-yellow and lilac-pink (P—p) are not yet clear. One might think both segregations to be caused by the same factor pair; but another possibility is that the difference white-yellow depends upon chromatophores and lilac-pink upon anthocyanine colour. If two such factor pairs are really involved, two explanations are possible: (a) chromatophore colour does not visibly change the anthocyanine-coloured flowers, (b) the factor for yellow chromatophore colour is (almost) absolutely linked with the factor for pink anthocyanine colour (p). In this latter case the above mentioned two plants with brownish lilac flowers could be explained as products of rare crossing-over. The question might be solved by studying the F_3 progeny, but this has hitherto not been possible because of the extremely poor fertility in almost all F_2 plants.

Length of flower. — The flower length (from the base of the calyx-like involucre to the tip of the perianth) was measured between two and four o'clock in the afternoon, i. e. one or a few hours before the flower has opened. This measured length nearly corresponds to the length of the flower tube after the opening. The measures are: *M. Jalapa* $\pm$ 40 mm., *M. longiflora* $\pm$ 140 mm. and $F_1 \pm 65$ mm. In F_2 the length ranged from 30 to 105 mm. Only one plant among the whole material had a flower length slightly over 100 mm. In 168 Swedish-grown F_2 plants the flower length was found to be as follows:

Length in mm:	35	40	45	50	55	60	65	70	75	80	85	90	Total
Number of plants:	2	13	12	42	45	27	12	8	3	2	2		168

This segregation may be explained by ascribing to *M. Jalapa* a number of more or less dominant factors for short flower. The possibility of a plasmatic influence, however, does not seem to be excluded.

Both parent species (section Eu-Mirabilis of the family Nyctaginaceae) have one flower within the calyx-like involucre. In F_2, however, a few plants often had three flowers within the involucre, thus resembling *Mirabilis triflora* of the section Quamoclidion.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Hairiness. — The leaves of *M. Jalapa* are smooth, the hairs are rather sparse and break off easily, they are bent and usually non-glandular (Fig. 20). *M. longiflora* (Fig. 21) is densely viscid-pubescent, its hairs are straight and the terminal cell is usually a typical, more or less globular gland-cell; the rich excretion causes stickiness of the leaves and flower-buds. The F_1 hybrid (Fig. 22) is densely pubescent, but non-viscid; the terminal cell of some hairs is slightly swollen, but no excretion is found [FUNAOKA (1925) studied hairiness in both species as well as the hybrid; in each of them he distinguishes between »Drüsenhaare» and »Deckhaare»]. Among all F_2 plants only one with rather sticky flower-buds was found. Most of these F_2 plants were examined under the microscope but not a single one with the typical glandular paternal type of hairiness was discovered. Thus, the type of F_2 segregation appears to be about the same as that for flower length and it evokes the same suspicion of a plasmatic inheritance.

III. CYTOLOGY.

In both species a somatic chromosome number of 58 was found. Within each genome there are extreme size differences between the chromosomes. The clearest pictures were obtained from *Mirabilis longiflora* (Figs. 1—6). In Fig. 1, a root-tip metaphase, four large and

Figs. 1—6. *Mirabilis longiflora* L. — Fig. 1, somatic plate with 58 chromosomes, including four large and two rather large ones. — Fig. 2, diakinesis in P. M. C., 29_{II}; two large pairs of »pseudo-univalents» close to the nucleolus. — Figs. 3—5, diakinesis, nucleolus with two adjacent large chromosome pairs; in Fig. 3 and 4 small satellites are visible. — Fig. 6, I—M, 29_{II}, three large pairs. — Figs. 7—9. *Mirabilis Jalapa* L. — Fig. 7, I—M, 29_{II}, one very large pair and two large pairs. — Fig. 8, diakinesis nucleolus with two large pairs attached. — Fig. 9, somatic prophase, nucleolus with the six large chromosomes attached. — Figs. 10—15. *M. Jalapa* × *M. longiflora* F_1 (Figs. 10 and 11 plant 7, Figs. 12—15 plant 10). — Fig. 10, diakinesis in P. M. C., $28_{II} + 2_I$?, the six large chromosomes close to the nucleolus, one heteromorphic pair among them probably as 2_I. — Fig. 11, diakinesis, nucleolus with two adjacent large pairs, one heteromorphic. — Fig. 12, I—M, 29_{II}, the heteromorphic pair at 11 o'clock. — Fig. 13, I—M, $28_{II} + 2$ large$_I$, the heteromorphic pair above the centre, with a large univalent to the left and one to the right. — Fig. 14, I—M, side view (separately drawn), 28_{II} visible. — Fig. 15, I—M, the three large pairs, one as 2_I. — Figs. 16—19, F_2 plants. — Fig. 16, I—M, the three large pairs and two smaller$_I$. — Fig. 17, I—M, the three large pairs, the heteromorphic pair as 2_I. — Fig. 18, I—M, side-view (separately drawn), 29_{II}, one large heteromorphic pair and some smaller. — Fig. 19, somatic chromosome with satellites, rarely visible. — Figs. 20—22. Hair types from the leaf margin. — Fig. 20, *Mirabilis Jalapa*, non-glandular hairs. — Fig. 21, *M. longiflora*, typical glandular hairs. — Fig. 22, F_1 hybrid, some hairs slightly glandular. — Figs. 1—19 $\pm 3100\times$ (Fig. 9 not accurately known), Figs. 20—22 $\pm 14\times$.

two rather large chromosomes can be distinguished from the rest. In Fig. 2, diakinesis in a P. M. C., the three largest pairs are easily recognized: two of them as »pseudo-univalents» adjacent to the nucleolus in Fig. 2 *a* and one as a ring bivalent in Fig. 2 *b*. Some pairs are extremely small and sometimes hardly visible. In Fig. 2 one clear ring bivalent is seen (in Fig. 2 *b*) and one rod (within the outline of the nucleolus), but in all other pairs connections could hardly be seen or could not be seen at all. Nevertheless, all pairs must be supposed to form real bivalents, since no univalents were found at first metaphase. In many of the cells at diakinesis one or two large chromosome pairs are attached to the nucleolus or are situated quite close to it (Figs. 2—5). Three such nucleoli with adjacent large chromosome pairs are represented by Figs. 3—5. In all three cases the two chromosomes of each pair are, contrary to Fig. 2, closely connected by one or more chiasmata. The small satellites with which chromosome pairs in Figs. 3 and 4 are attached to the nucleolus were but rarely visible. In somatic cells they were seen only in a few F_2 metaphase plates, in one or two of the large chromosomes (Fig. 19). First metaphase always showed 29 bivalents (Fig. 6).

Mirabilis Jalapa (Figs. 7—9) shows the same features as *M. longiflora*. Among the 29 metaphase bivalents in Fig. 7 one very large pair and two large pairs may be distinguished, but in some other cells that was rather difficult. In *M. Jalapa*, just as in *M. longiflora*, one or two large chromosome pairs often lie close to the nucleolus, sometimes even all three. Fig. 8, diakinesis in a P. M. C., shows two pairs attached to the nucleolus, while in Fig. 9, a root-tip prophase, all the six large chromosomes are attached to it.

Reduction division in the F_1 hybrid (Figs. 10—15) is rather regular. Diakinesis has about the same appearance as in the parent species. In some of the F_1 pairs, however, the partners show a different contraction (Fig. 10, between 4 and 7 o'clock). In Fig. 10 the six largest chromosomes lie close to the nucleolus, in Fig. 11 four of them. In first metaphase plates of the F_1 hybrid (Figs. 12—14) the three large chromosome pairs may be recognized rather easily, in Fig. 13 one pair as univalents. One of the large pairs is highly heteromorphic, consisting, in all probability, of a very large *Jalapa* and a rather large *longiflora* chromosome (in Figs. 12—14: at 11 o'clock, above the centre and as No. 13 from the left respectively). Some of the smaller pairs were also slightly heteromorphic. In Fig. 15 only the three large chromosome pairs are represented. In both diakinesis figures (Figs. 10 and 11)

the heteromorphic pair lies close to the nucleolus, in Fig. 10 probably as two univalents.

In F_1 plant 7 (mother plant to F_2) no first metaphase plates could be studied, but in F_1 plant 10 nineteen plates could be analysed more or less completely, with the following result:

Completely analysed	Less completely analysed
5 cells 29_{II}	2 cells.... $+ 0_I$
4 » $28_{II} + 2$ large$_I$	1 cell .... $+ 2$ large$_I$
4 » $28_{II} + 2$ small$_I$	1 » .... $+ 2$ small$_I$
1 cell $27_{II} + 2$ large$_I + 2$ small$_I$	1 » .... $+ 2$ large $+ 1$ small$_I$

Thus, in many cells, one large pair is found as two univalents. The large heteromorphic pair, on the other hand, seems almost always to form a bivalent. In the same F_1 plant 10, four or five out of six first anaphases showed some irregularities and in at least three of the cases large chromosomes were involved.

Some F_2 plants were also studied. Of 26 plants, 12 were found to have probably 58 chromosomes, while in 10 further plants the number was 58 or one chromosome more or less; two plants had probably 57 and one 59 chromosomes. One plant, finally, was triploid or subtriploid, with 85 to 87 chromosomes. Reduction division was studied in 13 F_2 plants (Figs. 16—18). Its general features are about the same as in F_1, though the frequency of univalents is not quite the same in all F_2 plants. In Fig. 18, first metaphase in a P. M. C., the three large pairs, one of them the heteromorphic, are easily seen. The members of some other pairs (two and thirteen from the right) are also different in size.

IV. FERTILITY.

The quality of the pollen was studied after staining with aceto-carmine-glycerine. The classification into »good» and »poor» grains (the former being coloured and having a more or less normal appearance of the protoplasm) was sometimes rather arbitrary. Five *Jalapa* plants had 97, 93, 92, 91 and 81 % good pollen. Seven *longiflora* plants had 97, 97, 96, 95, 94, 68 and 59 %.

F_1 plant 7 (mother plant of F_2) showed 38 % good pollen and F_1 plant 10 15 %, but, unfortunately, the number of counted grains in both plants is too low. In one flower of »*Jalapa* 93 %», of »*longiflora* 94 %», of »*longiflora* 59 %» and of »F_1 15 %» the pollen grains in

every loculus were studied and counted. The results were in good agreement with the above-mentioned percentages of good pollen. From the counts it appeared that the number of P. M. C's in these loculi varied from 7 to 11. The average number per loculus was: *Jalapa* 8,2, *longiflora* 8,8 and 9,0, F_1 8,4.

In 77 F_2 plants the percentages of good pollen were:

Percentages	0	10	20	30	40	50	60	70	80	90	100
Number of plants	26	19	16	4	4	5	2	1			

Thus, most of the F_2 plants had a lower pollen fertility than their F_1 mother plant (38 %).

Seed setting in the pure species is very abundant. The F_1 hybrid shows a rather reduced fertility. In the first year, when each branch was enclosed in an isolation bag, no fruits were produced. Later on, after free pollination but spatial isolation, F_1 plant 7 produced some hundreds of seeds yearly. In F_2 seed setting is extremely poor. Some plants die at an early stage, and in others the flower-buds do not develop. The majority of the F_2 plants, however, flowered abundantly, but most of these normally flowering plants produced no seeds or less than five, and plants with more than twenty seeds were very rare.

V. DISCUSSION.

The cross *Mirabilis Jalapa* L. × *longiflora* L. shows characteristics analogous to those of many other species crosses. (*a*) A perfectly clear Mendelian segregation for some alternative flower colour factors. (*b*) A polymeric segregation for quantitative characters such as intensity of flower colour (probably three factor pairs), length of flower and type of hairiness. (*c*) An extremely complex segregation for growth habit, which is not at all surprising, as the very typical habitus of both parent species depends upon numerous characters. In these types of segregation no essential difference from variety crosses need be seen (cf. OEHLKERS, 1942).

Though more than one thousand F_2 plants were studied, not a single one showing the pure paternal (*longiflora*) type as regards length of flower or type of hairiness was found. This may be explained by the highly polymeric character of the segregation, but it might also be caused by a plasmatic influence of *Mirabilis Jalapa*. A study of the reciprocal cross would elucidate this point, and the efforts to realize the cross (pollination at young stages, transplantation of a *Jalapa* style,

etc.) will be repeated. In this connection, it may be remembered that the great length of the *longiflora* style is not the only factor which prevents the fertilisation with *Jalapa* pollen, for JOST (1907) found that, in the *longiflora* style, the *Jalapa* pollen tube ceases to grow before it has reached the length of the *Jalapa* style.

In agreement with SHOWALTER (1935) the somatic chromosome number was determined to be 58. Among the 29 pairs of *M. longiflora* two large pairs and one rather large could be distinguished (according to SHOWALTER: one large and one rather large), and among those of *M. Jalapa* two large pairs and one very large (according to SHOWALTER: one large pair). Often one or two of the large pairs (or even all three, at least in *M. Jalapa* and F_1) are connected to the nucleolus or are lying close to it, and in a few cases small satellites were seen in two of them. TISCHLER (1908, 1929) does not discuss the question of nucleolus-forming chromosomes, but his Fig. 9 (1929; *M. Jalapa*) very clearly shows two large pairs close to the nucleolus. SHOWALTER (1935), again, finds that generally two, and sometimes three, chromosomes (or groups of chromosomes) are attached to the nucleolus, but he does not always recognize very large chromosomes in them. According to SHOWALTER, at diakinesis chromosome pairs are attached end to end into groups (cf. his Figs. 9—15), an arrangement not occurring in my own material. Now, it seems possible that SHOWALTER may have misinterpreted large chromosomes attached to the nucleolus as groups of small chromosomes. The number of chromosomes in *Mirabilis* and related groups and especially the presence of three pairs of large nucleolus chromosomes suggest the probability of (secondary) polyploidy in *Mirabilis* (cf. TISCHLER, 1928).

At diakinesis the partners in the gemini usually lie beside each other without visible connection. In TISCHLER's (1929) Fig. 9 and in SHOWALTER's Fig. 12 some pairs show the same relation, though less extreme than in my own material. Perhaps my figures represent a somewhat later stage, but external conditions or fixation may have some influence also. From the literature TISCHLER (1943, p. 440) cites some other cases of gemini without visible connections.

Chromosome pairing in F_1 is rather regular: often 29 bivalents occur. Even the one highly heteromorphic large pair almost always forms a bivalent. In spite of the size difference in this and in some other pairs, indicating major structural differences between the parent species, no clear cases of multivalent chromosome configurations were seen. Analogous cases are represented, e. g. by WINGE's (1938) cross

of *Tragopogon pratensis* (n = 6) × *T. porrifolius* (n = 6) and by AVERY's (1930) cross of *Nicotiana longiflora* (n = 10) × *N. alata* (n = 9). In the first case the structural differences seem to be small and pairing is quite regular. In the *Nicotiana* cross, however, the differences are very striking and seem to affect every chromosome. Nevertheless, in 31 of the 52 cells studied the configuration at first metaphase was $1_{III} + 8_{II}$ or $9_{II} + 1_{I}$ and only once was a cell with two trivalents found. Lack of multivalent configurations may depend upon various structural peculiarities: size of chromosomes, position of centromere, size of translocated or duplicated segments, number and localisation of chiasmata. But, analogous to polyploids, some other imperfectly known factors may be of importance (DARLINGTON, 1937, differential affinity; MÜNTZING and PRAKKEN, 1940, tendency to two-by-two pairing). The earlier meiotic stages in *Mirabilis* deserve a thorough reinvestigation for many reasons: chiasma formation and the extreme terminalisation, attachment of chromosomes to the nucleolus, and in F_1 the regular pairing of the heteromorphic pair and the non-pairing of one other large pair.

Within the space of the present article a detailed discussion of the fertility relations is impossible. In my material, contrary to that of CORRENS and SHOWALTER, the quality of the pollen appeared to be very good in most, though not all, plants of the parent species. Further, meiosis in my material appeared to be quite regular in both species. SHOWALTER (1935), on the other hand, with respect to *M. Jalapa, longiflora* and their hybrid, states that »one type seems to be as irregular as another».

Fertility in the F_1 hybrid is rather reduced. In almost all F_2 plants fertility seemed to be reduced still more, most of them producing no or very few seeds. An opposite relation is found in the above-mentioned *Tragopogon* cross (WINGE, 1938), which shows a rather reduced fertility in F_1 but quite normal fertility in F_2 and the following generations. However, as to *Mirabilis*, it must be taken into account that the fertility of first year F_2 plants was compared with that of old F_1 plants and that fertility in the latter may have increased in the course of years, a phenomenon that has been observed in many hybrids.

(Thanks to the kindness of Professor J. A. HONING and Mr. J. JANSEN at the Laboratory of Genetics in Wageningen two new F_1 plants were obtained and studied there. While reading the proofs of the present paper, I was informed that these new F_1 plants in their first year (1942) after free pollination produced 111 and 75 fruits, i. e.

much more than any first year F_2 plant. In the second year (1943) their production was not more than in the first year, on the contrary, much less, viz. 19 and 25 seeds respectively, a reduction that was probably caused by the cold and rainy summer and (possibly) by a better spatial isolation. At any rate, the better fertility of first year F_1 plants, as compared with the first year F_2 plants, seems to be quite evident.)

SUMMARY.

1. The somatic chromosome number in *Mirabilis Jalapa* and *M. longiflora* is 58. In both species three of the chromosome pairs are large or rather large. During prophase one, two or all three of these long pairs are attached to the nucleolus or lie close to it.

2. In the hybrid (*M. Jalapa* × *longiflora*) 29 bivalents often occur at first metaphase, but in most cases two or four univalents are present. Of the three large pairs one is clearly heteromorphic and some of the smaller pairs are slightly heteromorphic.

3. Fertility in the F_1 hybrid is rather highly reduced. In F_2 almost all plants, though having 58 or about 58 chromosomes, are still more sterile than F_1. One triploid F_2 plant was observed.

4. In F_2 some flower-colour factors show a perfectly regular Mendelian segregation: with anthocyanine versus without and (in the first ones) lilac colour versus pink. The intensity series of the anthocyanine colour probably depends on segregation of three intensity factors which are present cryptomerically in the yellow-flowering *Mirabilis Jalapa*.

5. Segregation for length of flower and type of hairiness seems to be controlled by polymeric factors. The pure paternal (*longiflora*) type of any of these two characters never occurred. This may be due either to a high number of factors or to plasmatic influence.

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AN EFFICIENT CHEMICAL FOR THE INDUCTION OF STICKY CHROMOSOMES

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DURING a study of the influence of organic compounds on mitoses, in which the effect of more than fifty substances has been investigated, I have been able to establish the fact that practically all these substances are able to induce either full or partial colchicine mitosis, or at any rate to influence the cell in the same way as colchicine, although the effect may be so weak in quantity that it cannot be called a disturbance. Thus, the ability to affect the cells in this way is of quite general occurrence. The results of these investigations will be described elsewhere.

Some chemicals were also found, in their highest concentrations, to lead to a more or less pronounced liquefaction of the chromosome substance. This usually occurred in such concentrations as were strongly toxic; so it was scarcely possible to decide whether the more extreme cases could be considered as truly vital. One important exception to this was found, however, viz. ethylene glycol, $HO \cdot CH_2 \cdot CH_2 \cdot OH$. This substance is quite unparalleled in its property to induce a strong liquefaction of the chromosomes in concentrations of only low toxicity.

The material on which the experiments were performed was root tips of *Allium Cepa.*

The most efficient concentration of ethylene glycol contained 1 mol per litre. The root tips submersed in this solution seemed to be alive even after eight days, at least their turgor still remained. Fixations were made after 4 hours, 12 hours, and 3 days. Mitoses were still going on after 3 days of treatment.

The experimental results are as follows:

4 mol solution. — After four hours the roots are partially slack. The structure of the resting nuclei is clearly coarsened. The chromosomes are highly liquefied and fuse to irregularly shaped masses, from which some chromosome arms protrude. It is rather dubious whether the cells are alive. After 12 hours the roots are quite slack and obviously dead; so no further fixations were made.

2 mol solution. — 4 hours: The root tips are slightly slack. The resting nuclei are quite normal in appearance. There are clear disturbances in development and function of the spindle (partial colchicine effect). A few anaphases show bridge formation owing to chromosome stickiness. 12 hours: The same symptoms, although more pronounced. 3 days: Root tips quite slack and obviously dead; no fixation was made.

1 mol solution. — 4 hours: Nothing abnormal was seen. 12 hours: Normal except for bridge formation in the anaphases. 3 days: There is a very strong liquefaction of the chromosomes. They fuse to irregularly shaped masses, where they are practically irrecognizable as individuals. From the liquefied »metaphase plate» there is flowing out jets of chromatin lengthwise in the spindle. In the anaphase and telophase there are numerous bridges between the chromatin lumps wandering apart. The spindle and the phragmoplast function normally; consequently two daughter nuclei separated by a cell-wall are formed, but the cell-wall is penetrated by fine chromatin threads connecting the two nuclei. The daughter nuclei after this type of mitosis are very easily recognizable by their shape and mutual position. It is beyond all doubt that the cells are alive during this process, as the chromatin is able to form daughter nuclei after mitosis, and the daughter nuclei are quite normal in structure (although, of course, abnormal in shape). No pycnotic nuclei or dead-looking cells were observed. A great proportion of the cells in the root tip have passed through this process; the frequency of the characteristic daughter nuclei is in at least some parts of the roots estimated at 20—30 %. It is, I think, rather remarkable that an influence which brings about such a severe change in the physical state of the chromatin does not kill the cells. There are, however, clear indications that the roots suffer from the ill-treatment. Thus, there was a considerable reduction in the frequency of mitoses in the three days fixation, and also an inhibition of the growth of the roots, but even after eight days they had not lost their turgor.

$^1/_2$ *mol solution.* — The fixations after 4 and 12 hours show nothing abnormal. The 3 days fixation shows a very clear liquefaction of the chromosomes, although the effect is by no means so strong as in the 1 mol concentration. The chromosomes do not fuse to a single shapeless mass but maintain their morphological individuality. The stickiness finds expression in the frequent occurrence of anaphase bridges. At this concentration there is only a slight reduction in the frequency of mitoses, and the roots continue to grow.

$^1/_4$ *mol and lower concentrations.* — No abnormal effects were seen.

Discussion. — Thus, ethylene glycol is able to induce partial colchicine disturbances in 2 mol solutions, and chromosome stickiness in $^1/_2$ mol and higher concentrations. The simultaneous occurrence of these two properties in one substance need not imply that they are causally related. The property of being able to induce spindle disturbances (c-mitosis) is present in most organic substances. Consequently, most substances having other conspicuous physiological effects will also, in addition to their more »specific» properties, be able to induce more or

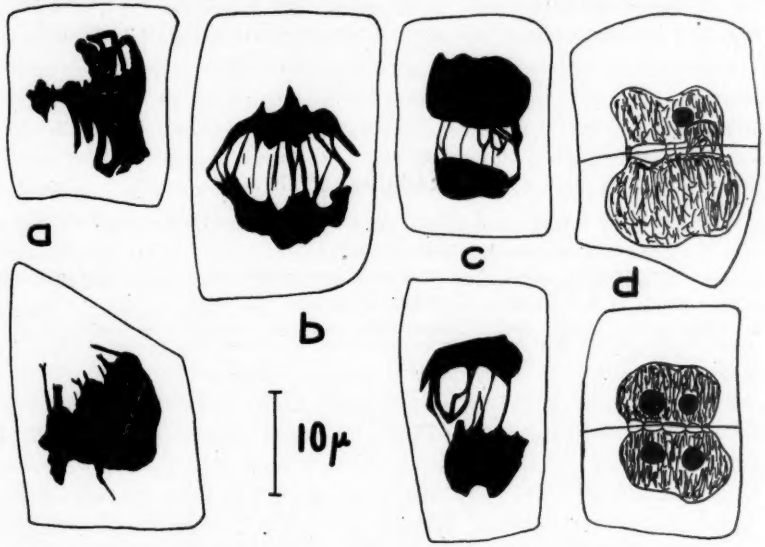

Fig. 1. Chromosome stickiness induced in root tip mitoses of *Allium Cepa* by treatment with 1 mol ethylene glycol for three days. — *a*, metaphase; *b*, anaphase; *c*, late anaphase—telophase; *d*, daughter nuclei.

less complete spindle disturbances. Many efficient c-mitotic substances do not give any chromosome stickiness in concentrations inducing full c-mitosis, and in ethylene glycol the chromosome stickiness was very pronounced in concentrations without visible spindle disturbances.

There are also other substances than ethylene glycol which are able to induce chromosome stickiness (e. g. ethyl alcohol), but in no other case seen by me was this property combined with such a low toxicity. As is well known, x-ray irradiation also makes the chromosomes sticky in a similar way. There is also known a recessive gene in maize (BEADLE, 1932, 1937) which changes the chromosomes in this way.

DARLINGTON (1942) suggests that these cases are due to a disturbed nucleic acid metabolism of the chromosomes.

Ethylene glycol is probably able to induce structural chromosome changes. Probably »gene mutations» would also appear in the progeny of treated plants. In the sticky chromosome plants of maize (BEADLE, l. c.) there is an increased frequency of both structural changes and mutations. It is possible that the mutations consist in minor changes in chromosome structure.

It would be interesting to try the substance on other organisms, e. g. to feed *Drosophila* with a medium containing ethylene glycol.

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NOTES ON THE CYTOLOGY OF DIPCADI AND BELLEVALIA

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DURING a study of the reaction of some Liliaceous plants to various c-mitotic chemicals I came across a species, *Dipcadi serotinum* MEDIC., which turned out to be of considerable cytological interest. First of all its chromosome number was as low as $2n = 8$, i. e. the lowest number reported for Liliaceae, and earlier found only in one other genus of the family, viz. *Bellevalia* (= *Hyacinthus* sectio *Bellevalia*). Further points of interest were the relation of the chromosomes to the nucleolus, the course of c-mitosis, and the chiasma formation at meiosis. As the cytological conditions were remarkably clear, the species must be considered a very favourable cytological object, worthy of future attention.

Owing to the rare occurrence of the chromosome number of *Dipcadi* I considered it appropriate to make a comparison with the cytological conditions of *Bellevalia*, both these genera belonging to the same subfamily of Liliaceae, viz. Scilleae. Fixations were therefore made of the three *Bellevalia* species grown at the Lund Botanic Garden: *Bellevalia romana* SWEET, *Hackeli* FREYN, and *Webbiana* PARL. During the study of one of these species, *Bellevalia Hackeli*, some interesting nucleolar conditions were encountered, which will also be described below.

All root chromosomes of this investigation were fixed in NAVASHIN, meiosis of *Dipcadi* was studied in smears fixed in BENDA or in permanent aceto-carmine slides. Most stainings were made in gentian violet. The nucleoli were studied also in slides stained with fuchsine and light green according to BHADURI.

I. THE CHROMOSOME MORPHOLOGY OF DIPCADI AND BELLEVALIA.

Root chromosomes of the two studied genera are pictured in Fig. 1 *a* and *b*. It is seen at the first glance that they represent two rather different types of idiograms, distinguished from each other both in chromosome size and morphology. The chromosomes of *Bellevalia*

romana have been studied earlier on several occasions. DARLINGTON (1926), DARK (1934) and MARQUARDT (1938) describe four types of chromosomes, and the same types are also found in *Bellevalia Wilhelmsii* by LEWITSKY (1931). The three species studied on the present occasion show concordant chromosome morphology with the earlier studied species. The following four types of chromosomes occur: A, the longest chromosome type (length about 15 μ), two-armed, with submedian centromere, the shorter arm with a small satellite; B (about 12 μ), sub-

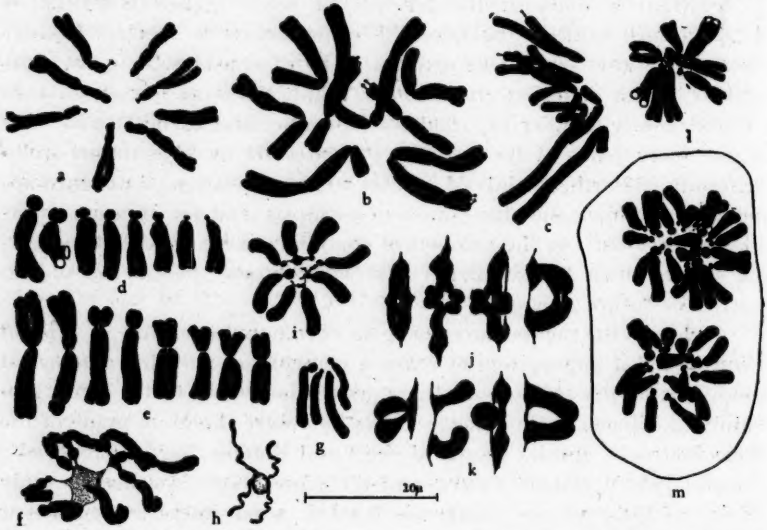

Fig. 1. *a, d, f—m*: *Dipcadi serotinum*; *b, c*: *Bellevalia Webbiana*; *e*: *Bellevalia romana*; *a, b*: somatic metaphase, *c*: one anaphase group in side view, *d, e*: c-mitosis at metaphase, *f*: somatic prophase, all chromosomes are in contact with the nucleolus, *g*: second meiotic anaphase, *h*: second meiotic telophase, *i*: somatic anaphase, *j, k*: two first meiotic metaphases, *l, m*: c-mitoses at metaphase. — × 2000.

terminal centromere, its longer arm is somewhat longer than the longer arm of A; C and D (9 and 8 μ) with submedian constriction. DARLINGTON and DARK found the shorter arms of B, C and D to be of about the same length. In my material this arm was decidedly shorter in B than in C and D. This was especially evident at the contraction during c-mitosis (Fig. 1 *e*), when the B chromosome had a rounded head, while the C and D always had cylindrical arms. The D chromosomes are furnished with a satellite on the shorter chromosome arms. This satellite was only occasionally visible in my material, while the A satellites were seen

regularly. The chromosome morphology is often especially clear at anaphase (Fig. 1 *c*).

The chromosomes of *Dipcadi* (Fig. 1 *a*) are smaller both in length and breadth than those of *Bellevalia*, and their morphology is different from that found in this genus. The four chromosome types may be designated A—D in order of diminishing size, as in *Bellevalia*, no homologies between the genera being implied by this, of course. All four types are subterminally attached, the shorter arm showing a decrease in size from A to D. After treatment with c-mitotic substances the short arms of all four types assume a spherical shape (Fig. 1 *d*), the A chromosomes having about the same proportions as the B of *Bellevalia*. The length of the chromosomes at somatic metaphase varies between 6,5 μ in A and 4,5 μ in D, thus less size variation within the idiogram than in *Bellevalia*. All the *Dipcadi* chromosomes are furnished with proximal differentiations: the B and C pairs have small satellites visible in most well-fixed plates, the A pair has a constriction on the shorter arm cutting off a rather large satellite, the short arms of the D pair are in themselves small and satellite-like.

The location of the centromeric constrictions of *Dipcadi* was often difficult to determine in untreated root metaphases, but at somatic anaphase (Fig. 1 *i*), at second meiotic anaphase (Fig. 1 *g*) and above all during c-mitosis (Fig. 1 *d*) the centromeric constrictions were quite evident. My experience is that at the routine study of chromosome morphology it may often prove helpful not only to study untreated material but also material submitted to a mild c-mitotic influence. The contraction of the chromosomes induced by this treatment makes it easy to find whole chromosomes lying in one plane, and their centromeric constrictions are exaggerated while secondary constrictions disappear.

II. THE ORIENTATION OF THE DIPCADI CHROMOSOMES AT NORMAL MITOSIS AND C-MITOSIS.

At somatic metaphase stage all chromosomes are with almost absolute regularity orientated so that all the shorter arms are turned inwards to the centre (Fig. 1 *a*). The chromosomes are arranged like the spokes of a wheel. This is the ordinary orientation found in most plants with subterminally attached chromosomes. During the progress of anaphase the chromosome heads gradually approach each other, and at late anaphase (Fig. 1 *i*) a characteristic picture is obtained. The chromosomes are decidedly more contracted than during metaphase,

and the staining of the heads begins to fade at about the same time as their outlines become less definite. Often, real connections are seen between the chromosome heads. In slides stained with light green it may be seen that the nucleolus is formed in the centre of the telophase plate. The chromosome heads are so close together at this stage that it is impossible to decide whether any particular chromosomes are involved in the nucleolus formation or whether all chromosomes take part in this process. At meiotic diakinesis usually two nucleoli are present, however, each of them joined to one bivalent. Maybe these two bivalents, which most stubbornly keep contact with the nucleolus, are those at which the latter is originally formed. During the mitotic resting stage usually only one nucleolus is found and the ordinary prophase picture shows the chromosomes to be strictly polarized, all the heads in one direction. Some of the chromosomes are very often attached at their heads to the nucleolus, in exceptional cases all of them may be connected to it as in Fig. 1 *f*. This condition is seen during meiosis, too, both at the interkinesis and at second telophase. The latter stage is pictured in Fig. 1 *h*.

This very characteristic behaviour during normal mitosis is still more accentuated during c-mitosis. The typical picture of c-metaphase after a short treatment with a c-mitotic substance (in this case α-monochloro-naphthalene) is represented by Fig. 1 *l*. All the heads of the chromosomes are gathered very close together. Since the spindle is lacking, the centromeres are not arranged in one plane but evidently on a small sphere, with the rest of the chromosomes turned radially out in all directions. This orientation is characteristic also of more advanced c-mitotic metaphases, where the chromosome number has been multiplied several times. In such cells there are one, two or more dense clusters of chromosomes, within each of which the chromosomes are arranged as the radii of a sphere (Fig. 1 *m*). Probably the formation of nucleolus during c-telophase serves as an orientating factor, maintaining the arrangement of the chromosomes from one c-mitosis to the next one. It follows, of course, that this arrangement is not absolute, one or two chromosomes often lying free from the great clusters. Still, it is quite striking to see how, in the absence of the spindle, the nucleolus may bring about a regular chromosome orientation. This situation is made possible by the low chromosome number and the position of all centromeres close to the chromosome ends, which allows all chromosomes to be cemented together at every telophase, thus going through the resting stage more or less connected to each other.

III. MEIOSIS OF DIPCADI.

Only first metaphase was studied in detail. The pollen mother cells of each pollen chamber in *Dipcadi* are rather few, so one disadvantage with the present material is that suitable meiotic stages are troublesome to obtain. Meiosis is rather sensitive to fixing. Some tolerably good fixations of metaphase I were obtained, however.

Four bivalents are formed. Since they are smaller in relation to the cell-size than is the case in *Bellevalia*, their chiasma conditions are easier to analyse. One peculiar condition struck the eye immediately, viz. the high frequency of ring bivalents. Considering the shortness of the proximal chromosome arms only rod bivalents were expected. Still, in 20 analysed cells the following frequency of rings was recorded:

Number of rings per cell:	1	2	3	4
» » cases:	3	4	10	3

Mean: 2,65 rings per cell. It is, of course, not certain that all these cases represent real ring bivalents. One chiasma on the long arms very close to the centromere together with one at the end of the long arms may simulate the appearance of a ring bivalent, if the proximal portions of the long arms are drawn out. Such bivalents occur in material with chiasma localisation. FRANKEL (1940) records this type from different *Fritillaria* species (Types 2c and s2c of his Fig. 1, l. c., p. 12). But no doubt a great proportion of the rings recorded in *Dipcadi* are real and have chiasmata on both sides of the centromeres. The first and third bivalent of Fig. 1 *j*, for instance, are a very common bivalent type, in which the connection at the short arms is quite evident. The last bivalents in Fig. 1 *j* and *k*, on the other hand, are possibly false ring bivalents.

The bivalents had one to three chiasmata. Five cells with a couple of univalents were among the 20 cells analysed, a fairly high percentage of failure of metaphase pairing. The following frequencies of chiasmata per bivalent were found:

Number of chiasmata per bivalent:	0	1	2	3
» » cases:	5	20	34	21

Mean: 1,89 chiasmata per bivalent. The chiasma frequencies counted per cell were:

Number of chiasmata per cell:	5	6	7	8	9	10	11
» » cases:	1	5	4	4	5	0	1

Mean: 7,55 chiasmata per cell. Of these 5,00 chiasmata were terminal and 2,55 interstitial.

The high frequency of chiasmata on the short arms cannot be explained on the basis of random distribution of chiasmata over the entire chromosome length. Some special condition possibly connected with the other differential properties of the proximal arms brings about a preponderance of the chiasmata to the short arms.

IV. NUCLEOLAR CYCLE OF BELLEVALIA HACKELI.

One of the *Bellevalias* investigated, viz. *B. Hackeli*, showed interesting nucleolar conditions. During mitotic prophase one or two large intranuclear nucleoli were present (Fig. 2 *a*). They persisted also after

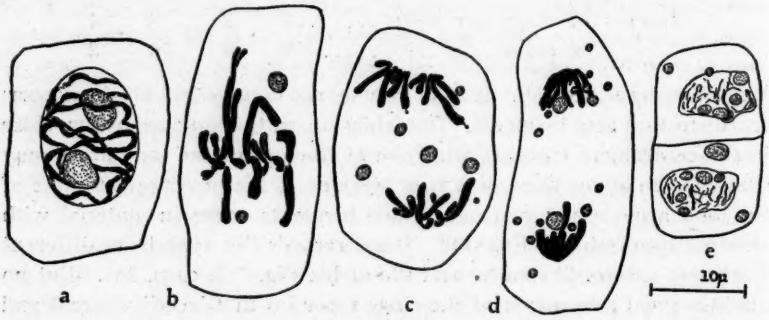

Fig. 2. *Bellevalia Hackeli*, the somatic nucleolar cycle, *a*: prophase, *b*: metaphase, *c*, *d*: anaphase, *e*: telophase. — × 1500.

the disappearance of the nuclear membrane, one, or as in Fig. 2 *b*, two nucleoli lying free in the plasm. They were ordinarily not attached to any chromosomes. At the division of the centromeres the nucleoli had disappeared, and there was a short nucleolus-free stage at early anaphase. At somewhat later anaphase, however, several extranuclear nucleoli were formed quite regularly (Fig. 2 *c*). They varied very much in size and number and were evidently formed by the separating anaphase chromosomes, as they were always lying at this stage between the separating anaphase groups. It could be directly seen in some cases how the nucleoli budded off from the ends of the anaphase chromosomes. At later anaphase (Fig. 2 *d*) the nucleoli were often scattered over the whole cell while some nucleoli were in clear contact with the chromosomes. At telophase (Fig. 2 *e*) nucleoli were still formed by the chromosomes, but from now on they were enclosed within the

nucleus, finally fusing to the one or two large nucleoli present during the resting stage. The extranuclear nucleoli already began to disappear at telophase, and during the resting stage the plasm gradually became free from nucleoli.

Thus, during the nucleolar cycle of *Bellevalia* two kinds of extranuclear nucleoli occur: the anaphase nucleoli, which are directly formed in the plasm, and the metaphase nucleoli, which are intranuclear in origin, but which remain after the nuclear membrane has disappeared.

There is little doubt that this behaviour of the nucleolus is quite parallel with that described for the meiotic nucleolar cycle of *Pisum* by HÅKANSSON and LEVAN (1942), which in its turn is a parallel with the *Fritillaria* case of FRANKEL (1937). FRANKEL, however, was not quite clear as to the nucleolar nature of his »telophase globules». In his work mainly gentian violet was used, which stains nucleoli and chromatin alike. Some of his slides were re-stained with fuchsine but failed to yield conclusive evidence on the critical point: »Whilst the telophase globules were found to be stained, this also was the case with the nucleoli, so that in *Fritillaria*, fixed with reagents containing osmic acid, this method apparently does not possess diagnostic value» (l. c., p. 44). There is evidently no reason, however, to regard *Pisum* and *Fritillaria* as contrary to each other as is to some degree implied by TISCHLER (1943, the foot note on p. 463). In *Pisum* as in the present *Bellevalia* case the nucleolar nature of the small bodies in question has been proved. They assume a clearly green colour in the BHADURI staining. It should be mentioned that during his studies of Taccaceae HÅKANSSON so far back as 1921 observed a frequent occurrence of similar telophase globules both at male and female interkinesis of *Schizocapsa plantaginea* (l. c., pp. 197 and 208, Figs. 13 and 28). The phenomenon was correctly interpreted as a presence of extranuclear nucleoli.

Bellevalia Hackeli, thus, is an instance of the occurrence at mitosis of a peculiar nucleolar behaviour that has previously been observed only at meiosis of a few genera and that has been studied in detail in the two cases *Fritillaria* and *Pisum*. The three genera concerned show the following similarities in nucleolar conditions: (1) persisting nucleolus (in mitosis, *Pisum*, *Bellevalia*; in meiosis, *Fritillaria*); (2) »cap» nucleolus at meiotic prophase (*Fritillaria* and *Pisum*; conditions of *Bellevalia* not known); (3) nucleolar telophase globules (in mitosis, *Bellevalia*; in meiosis, *Fritillaria* and *Pisum*, in the latter also in mitosis in exceptional cases). A condition for the origin of the telophase globules seems to be that a large amount of nucleolar material is formed and that several

chromosomes may participate in the nucleolus formation. In *Pisum* it was directly observed that each of the chromosomes normally forms nucleoli, strangely enough not at the satellites but at the centromeres.

SUMMARY.

1. *Dipcadi serotinum* is a new instance of a Liliaceous plant with the chromosome number $2n = 8$.

2. Its chromosome morphology is described in comparison with the conditions in three *Bellevalia* species with the same chromosome number.

3. The orientation of the chromosomes of *Dipcadi* at metaphase of mitosis and c-mitosis seems to be caused by the fact that all chromosomes may be attached to the nucleolus during interphase.

4. Meiosis of *Dipcadi* is described.

5. *Bellevalia Hackeli* forms nucleolar »telophase globuli» during mitosis.

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EXPERIMENTALLY INDUCED CHLOROPHYLL MUTANTS IN FLAX

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IN April, 1941, seeds of diploid and tetraploid material of three commercial varieties of flax, viz. Herkules, Concurrent and Palermo, were treated with X-rays. In each variety three diploid and three tetraploid seed portions of 100 seeds were treated. Three dosages were given, viz. 15000, 25000 and 35000 r, the X-ray intensity being 2500 r per minute. The seeds were dry when treated, and they had not been subjected to any pretreatment.

15 seeds of each portion were immediately put to germination. No especial reduction of the germination could be observed in the treated seeds. 20 seeds of each diploid portion and 85 seeds of the tetraploid portions were sown in pots in the greenhouse for later transplantation into the field. After being planted in the field the tetraploids of Herkules and Concurrent turned out to be decidedly less damaged by the X-radiation than the diploids. This is to be expected, since weak points occurring in the chromosomes are more easily compensated in a tetraploid cell than in a diploid cell. The following percentages of the plants survived the transplanting into the field:

X-ray dosage		15000 r	25000 r	35000 r
Herkules	diploids	62,5	10,5	10,0
	tetraploids	80,7	79,0	64,9
Concurrent	diploids	100,0	38,9	15,4
	tetraploids	82,6	86,2	72,3

This difference did not appear in Palermo, in which variety no significant lethality at all was met with.

In 1942 progenies were sown of the following number of X_1 plants:

	2x	4x
Herkules	13	62
Concurrent	17	80
Palermo	19	43
Total	49	185

These progenies were distributed over the three treatments in the following way:

	2x	4x
15000 r	26	69
25000 »	12	56
35000 »	11	60

In most of the progenies there were about 100 plants, but in the so-called B-series of the tetraploids, which consisted of progenies of 94 especially weak and badly damaged X_1 plants, there were only 30 plants in each family.

In this X_2 material three families were detected which segregated in a chlorophyll deficiency. All of these three families were diploid. In accordance with previously found instances (e. g. in barley; MÜNTZING, 1942) no chlorophyll mutants occurred within the tetraploid flax material. The material present in 1942 was distributed as follows:

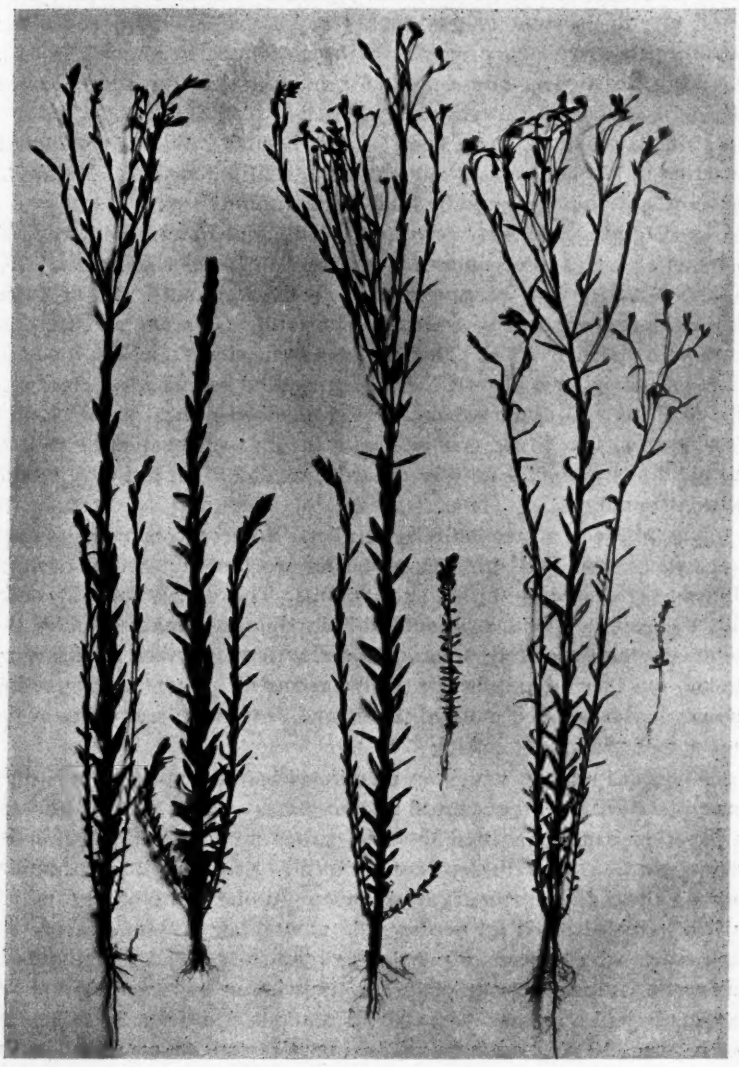
	Number of families	
	With one mutation	Without mutation
Diploids	3	46
Tetraploids	0	185

All three mutations occurred in families the mother plant of which had been treated with the lowest X-ray dosage. One mutation was among the Herkules material, the two others were among Concurrent.

The appearance of the mutants may be seen from Fig. 1, where one typical mutant has been pictured beside a normal plant of the same family. The most viable mutant (hereafter called mutant 1) is a *virescens* type which appeared in Concurrent. Its colour is yellowish-green. Its development proceeds more slowly than in the normal plants. Eventually it grows to the same height as the normal plants. It passes through the flowering stage and sets abundant seed.

Mutant 2, also occurring in Concurrent, is only slightly lighter green than normal Concurrent. Its growth is checked at an early stage, however, and it never reaches a greater height than 40 to 50 cm. The leaves are thin and narrow and its general appearance is very slender. It is easily distinguished from other more casually occurring dwarfed plants. It may survive until flowering, and may even form solitary seeds.

Mutant 3 of the Herkules variety is a purely whitish-yellow *xantha* type, which under field conditions never survives the seedling stage.



a *b* *c* *d* *e* *f*

Fig. 1. The appearance of the three mutants, *b*: mutant 1, *d*: mutant 2, *f*: mutant 3; *a*, *c* and *e*: normal sister plants.

This type always dies after 5 to 7 leaf-pairs are formed. If it is cultivated in the greenhouse and is protected from direct sunlight, its colour becomes pale-green and it may grow up to 50 cm. height. It leads a languishing existence, however, and I have not so far succeeded in keeping it alive until flowering.

Mutant 1 and 2 appeared in the approximate ratio 3 : 1, mutant 1 occurring in 17 plants among 70, mutant 2 in 21 among 118. Mutant 3, on the other hand, appeared in 3 individuals among 106.

In 1943 progenies were sown both of the diploid and the tetraploid X_2 material. The three mutant types were followed also through X_3. Mutant 1 was grown in progenies from 61 X_2 plants. 23 of these progenies were constantly green, 16 constantly *virescens* and 22 progenies segregated in 3 : 1. This deviates somewhat from the expected 1 : 2 : 1 ratio ($\chi^2 = 6{,}34$). In the 22 segregating families the class distribution was (all families being added together): 1233 green plants : 374 *virescens*. This fits in very well with the 3 : 1 segregation ($\chi^2 = 2{,}56$) and doubtless mutant 1 may be regarded as due to an ordinary monofactorial mutation.

Mutant 2 was grown in 95 X_3 families. No progenies of the mutant homozygote were present. 31 progenies were constant and normal, while 64 segregated in normal and mutants. This agrees almost exactly with the expected 2 : 1 relation. Within the segregating families the mutants were constantly somewhat deficient in number, which may depend on their dying before being recorded. The 64 segregating families had in all 3442 normal plants and 948 mutants. Owing to the shortage of mutants χ^2 is high (27,15).

The third mutant, which, as mentioned before, appeared in X_2 in a somewhat deviating frequency, 3 *xantha* plants among 106, also showed in X_3 other conditions than the two earlier mutants. 99 X_3 families were grown. 76 of these were uniformly green while 23 families showed a variating frequency of mutants. In one group of 12 families, each of which had on an average 78,2 plants, one mutant was present per family, which means a percentage frequency of 1,28 % mutants in this group. A second group of 11 families with an average of 75,7 plants per family had a greater frequency of mutants, i. e. from 3,8 to 26,9 %, mean value 13,09 % mutants. The material can be arranged in the following way:

Percentage of mutants	0	1,2—2,0	3,8—10,6	13,0—26,9
Number of families	76	12	5	6

The above four groups should, if a trifactorial segregation is assumed, represent the four segregation types: constant, 63 : 1, 15 : 1 and 3 : 1. The agreement with the expected relation of these four types (37 : 8 : 12 : 6) is rather bad ($\chi^2 = 16{,}95$). A great excess of non-segregating families and a deficit of 15 : 1 segregators are the main contributors to the χ^2. It should be observed that the original X_2 distribution agrees well with the 63 : 1 relation ($\chi^2 = 1{,}12$).

The assumption of a bifactorial segregation means no improvement, however. The second of the above groups would then represent the 15 : 1 segregations, while the two last groups would represent the 3 : 1 segregations. The agreement with the expected 7 : 4 : 4 distribution is very bad ($\chi^2 = 36{,}06$). The X_2 segregation 103 : 3 may very well be 15 : 1 ($\chi^2 = 2{,}12$).

The possibility of the segregation being monofactorial, which *a priori* would have been the most probable, seems to be quite unreasonable in this case. Thus, for instance, only 23 of the 99 families segregated and on the assumption of a 3 : 1 segregation, 66 families would have been expected to segregate. Further, the distribution of mutants over the X_3 families strongly suggests at least two distinct classes: 12 families with only 1 mutant and 11 families with between 4 and 21 mutants.

At the present stage of the investigation it does not seem very profitable to discuss any detailed auxiliary hypotheses in order to explain the behaviour of mutant 3. I shall only mention that one cytological change induced by the X-radiation may cause heterozygosity for two or even three homologous loci (this explanation has been suggested by Mr. ÖSTERGREN). If in one chromosome pair with a gene necessary for normal chlorophyll development this gene is translocated to a non-homologous chromosome, two chromosome pairs will be heterozygous for the gene, and the plant will segregate in 15 : 1. If the translocated segment should divide during its existence as a free fragment and each half be attached to two different non-homologous chromosomes, a 63 : 1 segregating plant may originate.

It must be pointed out that there is a great deficit of recessives. This deficit is greatest if a 3 : 1 segregation is assumed; calculated from the total number of recessives expected it will amount to 1131 plants. On the assumption of a 15 : 1 or a 63 : 1 segregation the deficit will be 505 or 162 plants respectively. Hence it is evident that a certain degree of lethality is active. The lethality may act on the gametic stage of the heterozygote, at an early embryonic stage, or at the germination of the

seed. The two latter possibilities can be discarded, since the number of capsules and the number of seeds are recorded for each X_2 plant and further the number of sown seeds is known in each X_3 family. No deficit is found either in the seed setting of X_2 or in the germination of X_3. Thus, the lethality must be gametic and acting in the pollen grains. This may explain the very great variation of the deficit between the families, recessives occurring in a frequency from 1,2 to 26,9 % in different cases. The growth of the pollen tubes may easily be subjected to great variations from environmental causes. Among GUSTAFSSON's (1938) chlorophyll mutations in barley six mutations showed a varying deficiency of recessives, which in all cases was shown to be caused by elimination of male gametes.

My thanks are due to Drs. GUSTAFSSON and TEDIN for valuable suggestions.

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CYTOLOGICAL STUDIES OF EXTRA FRAGMENT CHROMOSOMES IN RYE

I. ISO-FRAGMENTS PRODUCED BY MISDIVISION

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IN rye the normal somatic chromosome number is fourteen, but in several cases rye populations have been found to comprise individuals with an extra pair of small chromosomes. The literature on such so-called 16-chromosome rye was reviewed in two previous papers (MÜNTZING and PRAKKEN, 1941; MÜNTZING, 1943). This was done on account of the observation of a plant with 16 chromosomes in each of the varieties »Östgöta gråråg» and »Vasa II». The mode of meiosis in one of these plants (the one from »Östgöta gråråg») was described by MÜNTZING and PRAKKEN (1941, pp. 278—279). The plant in question had an extra pair of small chromosomes in addition to the seven normal bivalents. At first metaphase the additional chromosomes formed a bivalent in about one third of the p. m. c. In the other cells they were present as two small univalents. Pairing between the additional and normal chromosomes was not observed. As described by MÜNTZING (1943), a progeny was raised from the »Östgöta gråråg» plant with $2n = 16$, the chromosome numbers in this progeny ranging from 14 to 16. By raising two further generations a material was obtained in which the number of extra chromosomes ranged from 0 to 6. In this material the effects of different numbers of extra fragment chromosomes on vigour and fertility were studied (l. c.). Fertility was found to be more influenced by the fragment chromosomes than the vegetative development. At any rate, the measurements demonstrated that the chromosomes in question are not inert as was supposed by some previous workers. On the other hand, considering the size of the fragments and the effects of whole extra chromosomes, it was concluded that the fragments are probably subinert, having a lower proportion of active genes than ordinary chromosomes. In the same paper it was also mentioned that the extra chromosomes in my material, just as in the plants studied by LEWITSKY (1931) and HASEGAWA (1934), represent a special type of

chromosome not present in ordinary rye plants with $2n = 14$. In contrast to the normal chromosomes, which are more or less V-shaped, the extra chromosomes have a subterminal centromere. By this character and by their small size the fragment chromosomes may easily be distinguished also in somatic plates. Such a plate is represented by Fig. 7 in the present paper, showing the root tip chromosomes of a plant with four extra fragments. These root tips were fixed in diluted chrome-acetic-formalin and stained with gentian violet. All other slides to be discussed in this paper were prepared in the same way.

In the present report the cytology of plants with one single fragment chromosome in addition to the normal chromosome complement will be described. In the first place such plants with $2n = 14 + 1$ f are of interest for the attempts to clarify the possible homology between the fragment chromosome and some segment or segments in the other chromosomes. If such homology exists, the fragment chromosome might be expected to be paired at I—M at least in some cells. However, no evidence of true pairing was observed, though such cases were especially looked for. Altogether, meiosis was studied in 13 plants with one extra fragment chromosome, and in all of these plants the fragment was always observed to be unpaired. Evidence from a total of 15 plants with two or more extra fragment chromosomes also shows, without exception, that there is no association between the fragments and the normal chromosomes. This strongly indicates non-homology, but in order to settle the question definitely it would be valuable to study meiosis in haploids with extra fragment chromosomes. Such material, however, has not yet been available.

When the slides of the plants with one extra fragment chromosome were being looked through, it was observed that the size of the fragment was not always the same. On the contrary, the thirteen plants studied were found to represent four sharply separated categories in this respect. Besides plants with normal fragment chromosomes, two categories with smaller fragments and one category with a larger fragment were observed. As will be shown below, there is reason to assume that the three new categories have arisen as a consequence of misdivision of the centromere.

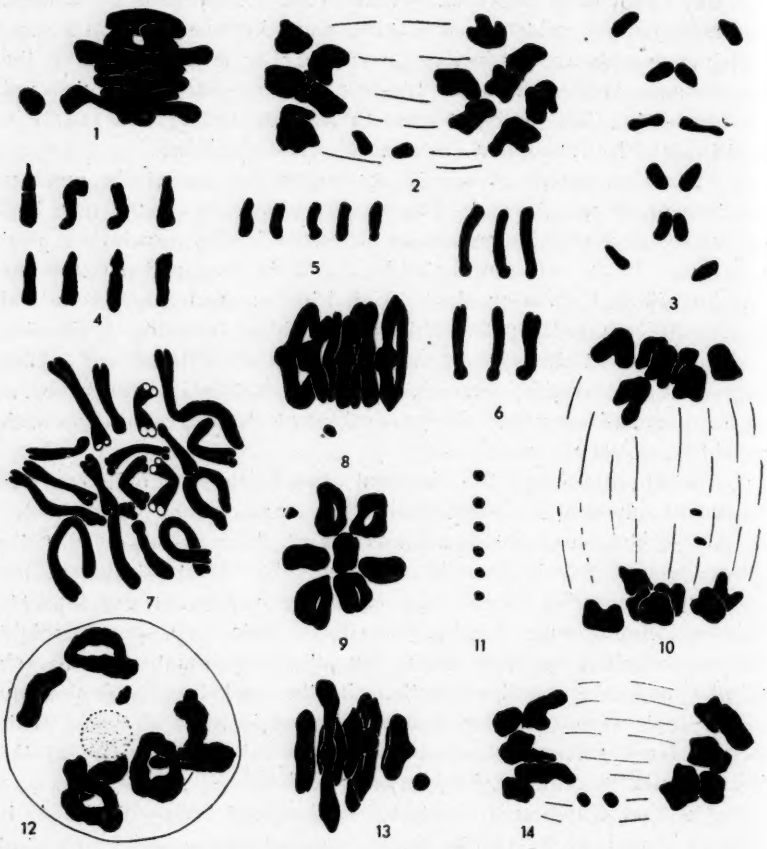
The normal, unchanged type of fragment chromosome is represented by Figs. 1—6. Fig. 6 shows its appearance in the root tips of two different plants, having $2n = 14 + 1$ f. The subterminal centromere separates a short head from a longer arm which is approximately

five times as long as the short, headlike arm. These somatic fragment chromosomes are evidently of just the same kind as those in Fig. 7, which represents a plant having $2n = 14 + 4$ f. They are also of the same general appearance as the fragment chromosomes of rye observed by LEWITSKY (1931) and LEWITSKY, MELNIKOV and TITOVA (1932) in root tips and by HASEGAWA (1934) in the pollen mitosis.

At first metaphase of meiosis the unpaired fragment chromosome has the appearance shown by Fig. 1. It is obviously smaller than half of any bivalent and the same size difference is also apparent at first anaphase. In the cell represented by Fig. 2 the fragment chromosome has just divided. The products of division are rod-shaped, one end leading the way to the pole. This is also evident from Fig. 3, showing divided fragment chromosomes at II—A from six different cells. The degree of stretching is different, but all the half univalents have the appearance and orientation to be expected of short chromosomes with a subterminal centromere.

The appearance of the fragment chromosomes at diplotene and second anaphase was also studied. The former stage is represented by Fig. 4, showing eight separate fragment chromosomes. They are all rod-shaped and in four of them the subterminal position of the centromere is rather clear. Fig. 5, finally, represents five separate fragment chromosomes lagging between the poles at I—A. As might be expected, they are now rather elongated and somatic-like, though smaller and more contracted than in the root tips. The position of the centromere is rather clearly indicated in most of them. This normal type of fragment chromosomes was observed in five of the thirteen plants examined, having $2n = 14 + 1$ f.

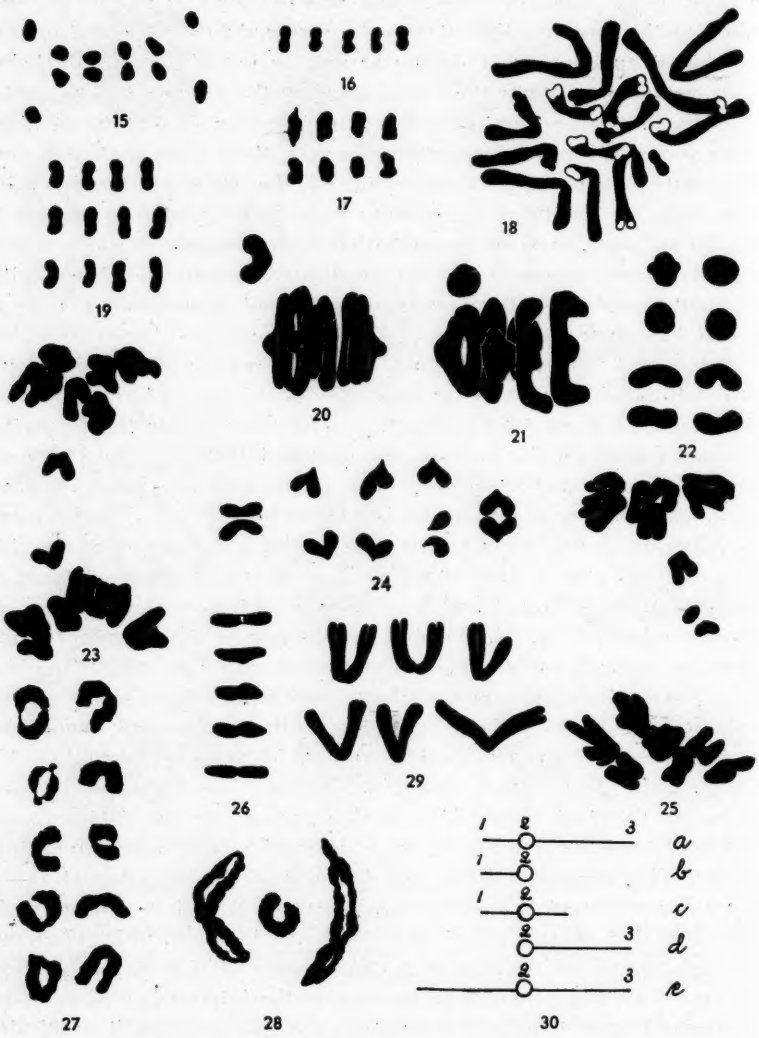
The first deviation was detected in the plant 1940—50—8. As is evident from Figs. 8—12, the single fragment chromosome present at meiosis in this plant was quite small. The size of this fragment is only a fraction of the size of the standard fragment, represented by Figs. 1—5. Nevertheless, this very tiny fragment chromosome showed just the same meiotic behaviour as the typical fragment chromosomes. Thus, it was observed to divide regularly at first anaphase (Fig. 10), and at second anaphase the products of division were seen to lag between the poles. These lagging half fragment chromosomes were still smaller than at I—M. Six laggards at II—A, from different cells, are represented by Fig. 11. The fragment could also be distinguished at prophase. Fig. 12 shows a nucleus at late diplotene with a small free fragment and seven ordinary bivalents. As the fragment is



Figs. 1—7, fragment chromosomes of the standard type at meiosis and mitosis. — Fig. 1, I—M with a free fragment; Fig. 2, division of the fragment at I—A; Fig. 3, divided fragments at I—A from six different cells; Fig. 4, eight separate fragments at diplotene; Fig. 5, five separate fragments, lagging between the poles at II—A; Fig. 6, fragment chromosomes from root tips of two different plants (one row of each); Fig. 7, somatic plate from a plant with $2n = 14 + 4$ f. — Figs. 8—12, meiosis in the plant 1940—50—8, having a small extra fragment chromosome. — Figs. 8—9, I—M in side view and polar view; Fig. 10, division of the fragment at I—A; Fig. 11, six separate fragments, lagging at II—A; Fig. 12, diakinesis with 7_{II} and a free fragment. — Figs. 13—14, meiosis in plants with a fragment of medium size. — Fig. 13, I—M; Fig. 14, division of the fragment at I—A. — × 2400.

present at all stages of meiosis and was observed in three different slides of the same fixation, it must be assumed to have a centromere capable of functioning at mitosis as well as meiosis. It would have been valuable to study the appearance of the fragment in somatic divisions. However, root tips of this plant had been fixed at the seedling stage and, besides the normal chromosomes, these roots contained *two* fragment chromosomes, these being of the standard type (as in Figs. 6—7). Though the possibility of an experimental error is not wholly excluded, it seems probable that in the somatic divisions of the plant in question one of the fragment chromosomes had been quite eliminated and the other one fragmented and eliminated to a large extent though not entirely.

The next category of fragment chromosomes was observed in four different individuals, the sister plants 1941—747—1, —11 and —15 and the unrelated plant 1942—290—2. In all these plants the fragment is intermediate in size between the standard fragment and the very small fragment just described. The appearance of these fragment chromosomes of medium size are illustrated by Figs. 13—19. A typical I—M group in side view with a free fragment is shown by Fig. 13. The medium size of the fragment is immediately apparent from a comparison with Figs. 1 and 8. The same is true of the fragment at first anaphase. Fig. 14, showing the division of the fragment chromosome under discussion, may be compared with Figs. 2 and 10. The half fragment chromosomes of the present category are more or less spherical at I—A, and the position of the centromere cannot be distinguished. This is evident from Fig. 14 and also from Fig. 15, showing the divided and separating fragment chromosomes at I—A from six different cells. At second anaphase, on the contrary, the lagging fragment chromosomes were observed to have a median centromere. This is evident from Fig. 16, showing the laggards at II—A from five different cells. The median position of the centromere is also indicated, though not clearly visible, at diplotene. Fig. 17 shows the appearance of the fragment chromosome at this stage. A comparison with Figs. 4 and 12 also reveals the intermediate size of the fragment chromosome under consideration. In this case it could also be studied in somatic divisions. In all four plants belonging to this category the fragment chromosome had the appearance shown by Figs. 18—19. Fig. 18 represents a complete metaphase plate with 14 normal chromosomes and one rather small fragment. The fragment appears to have a median centromere. This is perfectly clear from Fig. 19,



Figs. 15—19, meiosis and mitosis in plants with a fragment of medium size (continued). — Fig. 15, divided fragments at I—A from six different cells; Fig. 16, five separate fragments lagging at II—A; Fig. 17, eight separate fragments from diplotene; Fig. 18, somatic plate with $2n = 14 + 1$ f; Fig. 19, somatic fragments of

showing separate somatic fragment chromosomes from three of the plants belonging to this category. The fragments of these three plants are also of the same size, though two of them seem to have slightly more contracted somatic fragments than the third plant.

The most interesting of the deviating types of fragment chromosomes was observed in the plants 1941—746—4, 1941—749—3 and 1942—292—2. The meiotic behaviour of these plants is illustrated by Figs. 20—28. The first plant belonging to this category was observed to have an unusually large free fragment at first metaphase. At first this was believed to be due to an unusually weak contraction of the chromosomes, differences in contraction at meiosis being rather frequent also in population plants of rye. However, a closer examination soon revealed that the free fragments at I—M were of two different types. The most frequent type is represented in Fig. 20. It is a rather big, rod-shaped but bent fragment with a more or less clear median constriction. In other cells the fragment is present as a *closed ring* (Fig. 21). At first metaphase, it is true, the opening of this ring is almost quite filled and only noticeable in favourable cases by a weaker colour in the centre of the discoid chromosome. However, in most cells at I—M it was possible to decide whether the univalent was open or closed. In Fig. 22 four separate fragment chromosomes of each type are represented. In two of the rings the chiasmata are not completely terminalized.

In the three plants carrying the large fragment chromosome under discussion the frequencies of open and closed fragment chromosomes at I—M were determined, the following result being obtained:

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three different plants (one row of each). — Figs. 20—29, chromosomes of plants with a large iso-fragment. — Fig. 20, I—M with an open fragment; Fig. 21, I—M with a ring-fragment; Fig. 22, 4 ring-fragments and 4 open fragments at I—M from 8 different cells; Fig. 23, division of the fragment at I—A; Fig. 24, division of the fragment at I—A in 5 different cells (the second pair from the right showing misdivision); Fig. 25, I—A with misdivision of the fragment; Fig. 26, 5 separate fragments, lagging at II—A; Fig. 27, pairing of the two arms of the iso-fragment at diplotene (from 10 different cells); Fig. 28, the iso-fragment at diplotene, surrounded by two normal bivalents of the same cell; Fig. 29, separate root tip chromosomes, probably representing the iso-fragment (from two different plants, one row of each). — Fig. 30, diagram of the different types of fragment chromosomes studied, a = standard fragment, b = telocentric fragment representing the short arm of a, c = the iso-fragment corresponding to b, d = telocentric fragment representing the long arm of a, e = the iso-fragment corresponding to d (the centromere indicated by a circle; at the right arm of c and the left arm of e a figure has been omitted, the correct denominations are 1—2—1 and 3—2—3 respectively). — × 2400.

	Open	Closed	Uncertain
1941—746—4	69	23	15
—749—3	93	16	6
1942—292—2	100	23	10
Total	262	62	31

According to these values the fragment chromosomes were on an average open in 74 per cent of the cells and closed in 17 per cent. In 8 per cent of the cells their type could not be distinguished with certainty.

The occurrence of ring-shaped fragment chromosomes was definitely established by a study of prophase. At diplotene they had the appearance shown by Fig. 27. Of the ten separate chromosomes drawn, three are quite clearly closed, in three other ones the attachment is weak or has just been broken. Also in most of the remaining chromosomes the two arms show a tendency to pair. In the three quite closed rings the position of the centromere could not be distinguished from the point of attachment between the two ends. Fig. 28 shows a separate and almost closed fragment chromosome surrounded by two normal bivalents from the same nucleus. The size difference is striking. The ring-shaped fragment chromosomes were also distinguished at diakinesis and were found to be about intermediate in appearance between those at diplotene (Fig. 27) and first metaphase (Fig. 21). Thus, the gradual contraction and apparent disappearance of the opening of the ring could be followed quite clearly.

At first anaphase the median position of the centromere was quite clearly revealed by the separating halves of the fragment chromosome. Fig. 23 shows a typical cell at I—A, in which the size of the fragment chromosomes may be compared with that of the normal chromosomes. Fig. 25 shows a cell at just the same stage. In this cell one of the halves of the divided fragment chromosome is typical but the other half has split up into two equal parts, the point of breakage evidently being the centromere. The same phenomenon and the same stage is also represented by Fig. 24, the second pair from the right. Fig. 24 shows five separate pairs of dividing fragment chromosomes at I—A. In all cases the centromere is median and leads the way to the pole. Fig. 24 should be compared with Fig. 3 in order to make clear the different position of the centromere and the size difference between the standard type of fragment chromosome and the present deviating type.

The median position of the centromere was also clearly visible at second anaphase. Fig. 26 shows five separate fragment chromosomes lagging between the poles at this stage. The upmost one is especially well stretched. In this chromosome the median centromere region was visible as a small achromatic zone. In somatic divisions the present fragment type, on account of its relatively large size, is rather difficult to distinguish with certainty from the smallest V-shaped chromosomes of the normal somatic complement. In the best plates, however, this may be done fairly accurately. Fig. 29 represents six probable fragment chromosomes from two different plants. At any rate, no fragments of the standard type were present in the root tips of the plants in question.

Though the four different categories of fragment chromosomes described above are very well separated, it seemed desirable to define these differences more precisely by measurements. Thus, the free fragments at I—M were drawn with a camera lucida, and then the average diameter of the drawings was determined with the following result (Table 1).

TABLE 1. *Average diameter of the fragment chromosomes at I—M.*

Plant number	Diameter 2,0	2,5	3,0	3,5	4,0	4,5	5,0	5,5	6,0	6,5	7,0	7,5	8,0	8,5	9,0	9,5	n	M
1941—747—18						2	7	1									10	5,20
» —742—11							1	2	—	5	2						10	6,50
» — » — 6									3	4	3						10	6,75
» —744— 7							1	3	6								10	6,00
Total.....						2	9	6	9	9	5						40	6,11
1941—747— 1					4	6											10	4,55
» — » —11					1	8	1										10	4,75
» — » —15					10												10	4,25
1942—290— 2					1	6	3										10	4,85
Total.....					16	20	4										40	4,60
1940— 50— 8	2	5	3														10	2,80
1941—749— 3												1	2	4	2	2	11	8,34

The standard type of fragment chromosome is represented by the plants 1941—747—18, 1941—742—11, 1941—742—6 and 1941—744—7. The average diameters in these plants range from 5,20 to 6,75 units with a total average of 6,11. In the category »medium size», represented by the plants 1941—747—1, —11, —15 and 1942—290—2,

the average values range from 4,25 to 4,85. The total average of this category is 4,60. The significance of the difference in size between the two categories is obvious and needs no statistical treatment. This is also true of the two remaining categories »small» and »large», represented by the plants 1940—50—8 and 1941—749—3. The average values of these plants are 2,80 and 8,34 respectively and are evidently significantly different from all the other mean values. Each unit in Table 1 corresponds to 0,244 μ. Thus, the true average diameter of the four categories will be 1,49 μ (»standard»), 1,12 μ (»medium»), 0,68 μ (»small»), and 2,03 μ (»large»).

Though the average differences between the four categories are quite clear, it should be observed that nevertheless there is a significant heterogeneity within those categories which are represented by more than one plant, viz. the »standard» and »medium» categories. In both these cases the mean squares for »between» and »within» series were compared and were found to give the v^2-values 22,50 and 12,61 respectively. Both these values demonstrate a significant heterogeneity (P smaller than 0,001), which is most pronounced in the »standard» category. This heterogeneity is probably caused by different degrees of chromosome contraction in different plants. Such differences are not rare among population plants of rye and may be expected to influence the size of the fragment chromosome as well. In all the plants of the »medium» category the somatic chromosomes were also controlled and found to be of the same type (Figs. 18—19). In the »standard» category somatic fragment chromosomes could be studied in three of the four plants and were all found to be typical (Fig. 6). Thus, the heterogeneity observed is certainly not a reflection of structural differences but must be due to different degrees of contraction.

INTERPRETATION AND DISCUSSION.

In the first place the data reported above demonstrate that the stability of the extra fragment chromosomes in rye is not complete, new types of fragment chromosomes arising in considerable frequency. Of the thirteen plants examined, having a single extra fragment chromosome, not less than eight had new types of fragments not present in the parents. However, the frequency of new fragment chromosomes seems to be much higher in plants with a single extra fragment than in plants with two or more extra fragment chromo-

somes. Thus, at the same time as the thirteen plants with $2n = 14 + 1$ f, seven plants of the same variety, having $2n = 14 + 2$ f, were examined and were all found to have normal fragment chromosomes of the standard type. Also in three plants, having $2n = 14 + 4$ f, the fragment chromosomes were normal. It may also be mentioned that in six plants with $2n = 14 + 2$ f belonging to another variety (»Vasa II») the type of extra fragment chromosome was unchanged as compared to the mother plant.

The high frequency of new types of fragment chromosomes in plants with $2n = 14 + 1$ f and the absence or low frequency of such changed chromosomes in plants with $2n = 14 + 2$ f may have something to do with the abnormal behaviour of the fragment chromosomes at the first pollen mitosis. HASEGAWA (1934) observed a frequent occurrence of non-disjunction at this stage, both halves of the fragment chromosome often being included in the generative nucleus. This non-disjunction gives rise to an excess of plants with $2n = 14 + 2$ f and a deficit of plants with a single extra fragment chromosome. Such abnormal variation was observed by LEWITSKY, MELNIKOV and TITOVA (1932) and also occurs in my material (MÜNTZING, 1943, p. 93—94 and unpublished data). DARLINGTON and UPCOTT (1941) studying the inert B-chromosomes in *Zea*, find that these chromosomes have probably a weaker centromere than the other chromosomes. This centromere, »although usually sufficient for its smaller size, sometimes fails to work in concert with the larger ones». The authors also suggest that the same weakness is responsible for the peculiar behaviour of the supernumerary chromosomes at the pollen mitosis in *Secale*. This view is also based on the hypothesis advanced by DARLINGTON (1939, 1940) that the centromere is a compound structure containing several »centrogenes». By misdivision of the centromere new centromeres may arise with a lower number of centrogenes. If the number is not too low and the new chromosome not too large, the new centromeres will function, though sometimes less effectively than the original centromere. In the latter case the centromere will have a tendency to misdivide again.

Thus, if the abnormal behaviour of the fragment chromosomes at the pollen mitosis in rye is an indication of a weakness of the centromere, which seems plausible, this weakness may also be expected to lead to an occasional misdivision of the chromosomes in question. Further, the two products of a misdivision are certainly less likely to pass to the same pole than the halves of an incompletely

divided fragment chromosome, in which the division of the centromere is delayed. Thus, according to this hypothesis most generative nuclei of the pollen grains in plants with two extra fragment chromosomes will receive two unchanged fragment chromosomes as observed by HASEGAWA (1934), but when only one fragment chromosome is included in the generative nucleus this will often be a product of misdivision. This would, indeed, account for the high frequency of new chromosome types in plants with a single extra fragment chromosome and for the absence of such alterations in the plants with two or four extra fragment chromosomes.

Now, the data obtained definitely prove that at least one of the three new fragment types obtained is a product of misdivision of the centromere. The evidence available even strongly indicates that all of them have arisen by this process.

The best case is represented by the large fragment chromosome, having a median centromere (Figs. 20—29). At diplotene and diakinesis the two arms of this chromosome were frequently found to be paired, and in about 20 per cent of the cells at first metaphase the fragment chromosome was present as a closed ring. Thus, this chromosome is undoubtedly an *iso-chromosome* (DARLINGTON, 1939, 1940), derived from a telocentric fragment, arisen by misdivision of the centromere. Undoubtedly it is derived from the long arm of the standard fragment chromosome. This is evident from the size relationships. The only other possibility would be that it had arisen from one of the normal chromosomes. In such a case, however, the iso-chromosome should be expected to pair not only with itself but also, in some cases at least, with the corresponding chromosome arm in the normal chromosome complement. Such pairing, however, never occurred, the iso-chromosome always being present as a univalent. In *Zea mays*, on the contrary, an iso-chromosome representing one of the arms of chromosome number 5 was observed to pair in some cells with both the normal chromosomes number 5 (RHOADES, 1940, p. 503). In this way a trivalent was formed with the iso-chromosome in the middle. In other cells this chromosome formed a closed ring, the two identical arms pairing with each other just as in our rye case. Thus, the total absence of trivalents in the rye plants containing the iso-chromosome in question clearly shows that it is not derived from a normal chromosome but from an additional fragment chromosome. It should be mentioned that all the three plants showing these large iso-chromosome fragments were descendants from crosses

in which the male parent had two extra fragments of the standard type. Of the female parents, one also had $2n = 14 + 2f$, one had $2n = \pm 14$, and the third mother plant (1940—50—8) had $2n = = 14 + 2f$ in the root tips but at meiosis only the single, very small fragment described above (p. 233). The parent plants of the three individuals having the large iso-fragment were all different and, thus, misdivision of the standard fragment must have happened more than once. So far this misdivision has not been directly observed, but it will be looked for especially in the pollen mitosis. The centromere of the new large iso-fragment may be expected to be still less stable than the centromere of the standard fragment. This is indicated by Figs. 24 and 25, showing cases of misdivision of the iso-fragment at first anaphase. In both these cases the division of the iso-fragment has resulted in three products, one unchanged iso-fragment and two telocentric fragments. These telocentric fragments represent half the iso-chromosome and correspond to the long arm of the original standard fragment.

It will be very interesting to study the stability of the iso-fragment and its possible derivatives in the offspring of the plants having the large iso-fragments. These plants were perfectly fertile and vigorous, and progenies from two of them were raised this autumn (1943). The quite unreduced fertility and vigour of the mother plants represent a final proof that the iso-fragment in question is derived from the sub-inert standard fragment chromosome and not from a segment of the normal gene-carrying chromosomes. In such a case a marked disturbance of the balance would have occurred. In the maize case studied by RHOADES (1940) the secondary trisomes were highly sterile in both the male and female flowers and were also abnormal in morphological respects.

Though the evidence is less conclusive than in the case of the large iso-fragment, it is highly probable that the remaining two types of new fragment chromosomes in rye are also products of misdivision of the centromere. Thus, the very small fragment chromosome (Figs. 8—12) probably corresponds to the short arm of the standard fragment. This is strongly indicated by the size relationships and by the fact that this small fragment evidently has a centromere. The position of this centromere must be terminal, a supposition which is not contradicted by the meiotic observations. Unfortunately, the appearance of the fragment could not be studied in the root tips, the plant in question, as pointed out above, having two normal standard fragments in these parts of

the plant. Neither is it possible to study the small fragment in the offspring. Before it was observed, the plant had been used as the female or male parent of three different progenies, but in none of these progenies was the fragment recovered.

There is reason to believe that the medium-sized fragment (Figs. 13—19) represents the iso-chromosome corresponding to the small, probably telocentric fragment just discussed. As is evident from its appearance in somatic cells (Figs. 18—19) as well as at second anaphase (Fig. 16), it has a strictly median centromere. Further, its size would correspond quite well to twice the size of the short arm of the standard fragment chromosome. This is especially clear from the somatic chromosomes. At meiosis the medium-sized fragment appears to be slightly larger than twice the size of the small fragment chromosome. It is possible, however, that this slight discrepancy is simply caused by the fact that the slides containing the small fragment were older and consequently more bleached than the other slides. If the stain is heavy, the chromosomes will appear to be somewhat thicker than in the opposite case.

In this case of a probable, quite small iso-chromosome fragment no closed rings were observed at diplotene. However, as such rings were in a minority even in the case of the large iso-chromosome fragment, they are certainly not to be expected in the present case. Possibly pachytene pairing between the two arms may occur, but such a pairing will be very difficult to observe. More data about the small iso-fragment will probably be obtained from studies of the offspring. Also in this case the fertility and vigour of the plants carrying the fragment were not diminished at all. Three of these plants were sister plants derived from a cross between a mother without extra fragments and a father having two extra standard fragments. The fourth plant having the small iso-fragment was produced in the following year from a different pair of parent plants. In this case, too, the mother had $2n = 14$ and the father $2n = 14 + 2\,f$. Thus, the same type of new fragment chromosome may arise repeatedly. This fact strongly supports the view that it is the result of a misdivision in the male parent.

The interpretation of the results obtained is summarized in a diagram (Fig. 30). By misdivision the standard fragment (*a*) may give rise to two telocentric fragments, one small (*b*) and one larger (*d*). Secondarily these telocentric fragments give rise to two types of iso-fragments, one of medium size (*c*) and one large (*e*). So far the larger

telocentric fragment (*d*) has not been observed in entire plants but was observed to arise secondarily in plants having the large iso-fragment. In the drawings submitted Type *a* is represented by Figs. 1—7, Type *b* by Figs. 8—12, Type *c* by Figs. 13—19, and Type *e* by Figs. 20—29.

Misdivision of the centromere and the production of iso-chromosomes have been observed by several workers and especially by DARLINGTON (1939, 1940). He analysed the phenomena cytologically in great detail and also pointed out the theoretical importance of the processes. The divisibility of the centromere is evidently of great interest from several points of view. It may increase our knowledge of the chromosome mechanics at mitosis and meiosis and it helps us to understand the origin of new idiograms (cf. LEVAN and EMSWELLER, 1938) and new aneuploid chromosome numbers. Cytological observations of a transverse division of the centromere were made before 1939 by UPCOTT (1937) in *Tulipa* and KOLLER (1938) in *Pisum*. As early as 1931 NISHIYAMA, styding the cytology of fatuoid oats, observed the fragmentation of a lagging univalent at the second telophase. »The fragmentation appeared usually to occur at a point where the spindle fibers are attached» (NISHIYAMA, 1931 p. 73). This observation, however, was not further discussed.

In recent years some new cases of misdivision have been reported. HÅKANSSON (1940 a and b) saw the phenomenon in a haploid plant of *Godetia Whitneyi* and in a mutant type of the same species. LEVAN (1942) observed misdivision at first anaphase in haploid rye and new telocentric chromosomes at the second division.

The first case of iso-chromosomes, resulting from misdivision, is the secondary trisomes in *Datura* (BELLING and BLAKESLEE, 1924). Another secondary trisome was described by RHOADES (1933) in maize. Originally these secondaries were believed to arise by an inversed crossingover, but now it is rather certain that they are products of misdivision. In maize a telocentric chromosome, representing the short arm of chromosome No. 5, and the corresponding iso-chromosome were studied in great detail by RHOADES (1938, 1940). In this case the telocentric chromosome is rather stable, but occasionally it yields progeny with an iso-chromosome. By genetic studies it could be definitely demonstrated that the iso-chromosome represents a doubling of the telocentric chromosome. Another case of an iso-chromosome was observed by DARLINGTON and THOMAS (1941) among the B-chromosomes in a *Sorghum* species. In *Triticum vulgare*

HÅKANSSON (1932) found a univalent which formed a closed ring at meiosis. As pointed out by several workers, this ring-univalent was probably an iso-chromosome. Ring-univalents at meiosis in *Triticum* were also observed by LOVE (1939). HUSKINS and SPIER (1934) and LOVE (1938) have reported a chromosome in *Triticum* with a terminal centromere due to the loss of one arm. This chromosome is probably again a product of misdivision. Interesting enough, genetic evidence has been obtained which indicates that even the Y-chromosome in *Drosophila melanogaster* originated as an iso-chromosome, though it has been changed secondarily (cf. DARLINGTON, 1940, p. 359).

Besides being split by spontaneous misdivision the centromere may in rare cases be divided into two efficient parts by means of irradiation. This has happened a few times in maize (MCCLINTOCK, 1932, 1938; RHOADES, 1936). In most cases, however, the telocentric chromosomes that have arisen by a transverse division of the centromere have a poor stability and are soon replaced by the corresponding iso-chromosomes. In maize the telocentric chromosome studied by RHOADES (1940) was not constant in the somatic divisions and gave rise to new types of fragments. The corresponding iso-chromosome, on the contrary, was found to be stable.

The extra fragment chromosomes in rye were found to be subinert (MÜNTZING, 1943) and resemble in this respect the quite inert B-type chromosomes in maize. Another similarity is the instability of the extra chromosomes in both species. Also in this respect, however, the extra chromosomes in maize are more extreme. RANDOLPH (1928) distinguished a series of diminutive B-chromosomes. All of them had probably arisen by fragmentation from an original B-type. DARLINGTON and UPCOTT (1941) also found several types of B-chromosomes and smaller so-called b-chromosomes in maize. The smaller types are supposed to arise by deletion from the larger B:s. The liability of deletion is supposed to be increased by the heterochromatic constitution of the B-chromosomes.

According to the data given in the present report new types of extra fragment chromosomes arise in rye. So far, however, the new types observed have all been found to be products of misdivision of the centromere. The stability and meiotic behaviour of these different types of extra fragments will be further studied. However, the main problem in this rye material is the origin and function of the extra chromosomes of the standard type. In the first place it is necessary to decide whether they are really heterochromatic, as there is reason

to suspect. Information on this point may be gathered from experiments with low temperature. Such experiments have been started and may be described in another report.

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ABSTRACTS — KURZE MITTEILUNGEN

ENAR ANDERSSON and OLOF TEDIN: The effect upon the mean and variability of the dependent variate of a selection according to the independent.

In pure and applied genetics, as well as in other branches of experimental biology, the calculation of coefficients of correlation and regression is often a valuable aid in the interpretation of the results obtained. These coefficients may be used in various ways, one of the most useful being the determination of the residual variance in the dependent variate. In many instances, e. g. when the independent variate is more readily available for measurements or may be more easily controlled in an experiment than the dependent one, it may be of interest to know how a selection of material, founded upon the data of the former, will influence the mean and the variation of the latter. When the correlation distribution is not wholly normal, no general method for the determination of this effect of selection seems available to the biologist interested in the statistical treatment of his data. A general method for truncated normal curves and correlation distributions has been shown by QUENSEL (1940). In a study of the effect of selection in a plant-breeding experiment, carried out by the present authors (TEDIN and ANDERSSON, 1943), one of us, ANDERSSON, had the opportunity to elaborate such a method, and as this method should also be of some interest in connection with other problems of genetics (ANDERSSON, 1943) it is here briefly described.

Just as the frequencies of individuals with different measurements of one character in a normally distributed population may be represented by the »normal curve», the frequencies of individuals of different combinations of two normally distributed variates may be represented by a surface, somewhat similar to the surface of a mole-hill. Its highest point will correspond to $\bar{x}$, $\bar{y}$, and the frequencies decrease gradually on all sides, asymptotically approaching 0. The intersection between this surface and any horizontal plane will form a circle, whose axes are parallel with the x and y axes if the variates are uncorrelated, but an ellipse with its axes at an angle to the x and y axes if they are correlated. In the latter case a perpendicular plane through any of the axes of the ellipse will divide the »mole-hill» into two equal parts. The volume between the surface and the horizontal plane representing the frequency 0 represents the total of the population, and if this volume is cut by any perpendicular plane, the two parts will represent the frequencies of individuals in the population on both sides of the value of x or y, through which the plane is laid. By determining the characteristics in regard to y in such a part of the population it is possible to assess the effect upon y of a selection in x. In such calculations the constants are so chosen that the total volume becomes 1.

If both the variates are completely normally distributed, the formula of the »correlation-surface» is (cf. YULE and KENDALL, 1937)

$$F(x,y)=\frac{N}{2\pi\cdot\sigma_x\cdot\sigma_y\sqrt{1-r^2}}\cdot e^{-\frac{1}{2(1-r^2)}\cdot\left[\frac{(x-\bar{x})^2}{\sigma_x^2}-\frac{2r(x-\bar{x})(y-\bar{y})}{\sigma_x\cdot\sigma_y}+\frac{(y-\bar{y})^2}{\sigma_y^2}\right]}$$

If the distribution of the variates is not wholly normal, without deviating too much from normality, the formula of the correlation surface may be written by a series, the first term of which is the formula of the normal uncorrelated surface, the following ones being different derivatives from this function multiplied by certain constants. If $F(x, y)$ is dissolved into its components, $\varphi(x)$, $\varphi(y)$, the general formula of the correlation surface (QUENSEL, 1938) is

$$F(x,y) = \varphi(x)\cdot\varphi(y) + \lambda_{11}\cdot\frac{\delta\varphi(x)}{\delta x}\cdot\frac{\delta\varphi(y)}{\delta y} - \frac{\lambda_{21}}{2!}\cdot\frac{\delta^2\varphi(x)}{\delta x^2}\cdot\frac{\delta\varphi(y)}{\delta y} - \frac{\lambda_{12}}{2!}\cdot\frac{\delta\varphi(x)}{\delta x}\cdot\frac{\delta^2\varphi(y)}{\delta y^2} -$$
$$- \frac{\lambda_{30}}{3!}\cdot\frac{\delta^3\varphi(x)}{\delta x^3}\cdot\varphi(y) - \frac{\lambda_{03}}{3!}\cdot\varphi(x)\cdot\frac{\delta^3\varphi(y)}{\delta y^3} + \frac{\lambda_{22}+2\cdot\lambda_{11}^2}{2!\cdot 2!}\cdot\frac{\delta^2\varphi(x)}{\delta x^2}\cdot\frac{\delta^2\varphi(y)}{\delta y^2} + \frac{\lambda_{31}}{3!}\cdot\frac{\delta^3\varphi(x)}{\delta x^3}\cdot$$
$$\cdot\frac{\delta\varphi(y)}{\delta y} + \frac{\lambda_{13}}{3!}\cdot\frac{\delta\varphi(x)}{\delta x}\cdot\frac{\delta^3\varphi(y)}{\delta y^3} + \frac{\lambda_{40}}{4!}\cdot\frac{\delta^4\varphi(x)}{\delta x^4}\cdot\varphi(y) + \frac{\lambda_{04}}{4!}\cdot\varphi(x)\cdot\frac{\delta^4\varphi(y)}{\delta y^4} \ldots\ldots \text{ etc.}$$

In this formula $\frac{\delta\varphi(x)}{\delta x}$ is the first, $\frac{\delta^2\varphi(x)}{\delta x^2}$ the second, derivative of the function $\varphi(x)$, etc. The constants before the different derivatives of the normal »correlation-surface» are certain coefficients λ_{ij}, so-called seminvariants (THIELE, 1931). All these seminvariants except λ_{10} and λ_{01} are independent of the point about which the moments are chosen (v. YULE and KENDALL, 1937).

By integration it is possible to show that if the total population is cut by a perpendicular plane through x_1 the distribution of y in the part between x_1 and ∞ will be

$$F_{x_1}(y) = \frac{\int_{x_1}^{\infty} F(x,y)\,dx}{\int_{x_1}^{\infty} F(x)\,dx} = \varphi(y) + \frac{\delta\varphi(y)}{\delta y}\cdot\frac{\int_{x_1}^{\infty}\left(\lambda_{11}\cdot\frac{\delta\varphi(x)}{\delta x} - \frac{\lambda_{21}}{2!}\cdot\frac{\delta^2\varphi(x)}{\delta x^2} + \frac{\lambda_{31}}{3!}\cdot\frac{\delta^3\varphi(x)}{\delta x^3}\right)dx}{\int_{x_1}^{\infty} F(x)\,dx} +$$
$$+ \frac{\delta^2\varphi(y)}{2!\cdot\delta y^2}\cdot\frac{\int_{x_1}^{\infty}\left(-\lambda_{12}\cdot\frac{\delta\varphi(x)}{\delta x} + \frac{\lambda_{22}+2\lambda_{11}^2}{2!}\cdot\frac{\delta^2\varphi(x)}{\delta x^2}\right)dx}{\int_{x_1}^{\infty} F(x)\,dx} + \frac{\delta^3\varphi(y)}{3!\cdot\delta y^3}\cdot$$
$$\cdot\frac{\int_{x_1}^{\infty}\left(-\lambda_{03}\cdot\varphi(x) + \lambda_{13}\cdot\frac{\delta\varphi(x)}{\delta x}\right)dx}{\int_{x_1}^{\infty} F(x)\,dx} + \frac{\delta^4\varphi(y)}{4!\cdot\delta y^4}\cdot\int_{x_1}^{\infty}\frac{\lambda_{04}\cdot\varphi(x)}{F(x)}\,dx + \ldots. \text{ etc.}$$

$$F(x) = \varphi(x) - \frac{\lambda_{30}}{3!}\cdot\frac{\delta^3\varphi(x)}{\delta x^3} + \frac{\lambda_{40}\cdot\delta^4\varphi(x)}{4!\cdot\delta x^4}$$

From this formula it is again possible to determine the difference between the total mean of y and the mean in the cut-off part of the population as well as the variance in y within the cut-off part. Let the former difference be m and the latter variance σ_{yr}^2. The formulae are

$$m = \sigma_y \cdot r \cdot \frac{\varphi(x) + \frac{\gamma_{21}}{2!} \cdot \frac{\delta \varphi(x)}{\delta x} - \frac{\gamma_{31}}{3!} \cdot \frac{\delta^2 \varphi(x)}{\delta x^2}}{\int_{x_1}^{\infty} \varphi(x) dx + \frac{\gamma_{30}}{3!} \cdot \frac{\delta^2 \varphi(x)}{\delta x^2} - \frac{\gamma_{40}}{4!} \cdot \frac{\delta^3 \varphi(x)}{\delta x^3}}$$

$$\sigma_{yr}{}^2 = \sigma_y{}^2 \cdot \left[1 + \frac{\gamma_{12} \cdot \varphi(x) - \frac{\gamma_{22} + 2\gamma_{11}^2}{2!} \cdot \frac{\delta \varphi(x)}{\delta x}}{\int_{x_1}^{\infty} \varphi(x) dx + \frac{\gamma_{30}}{3!} \cdot \frac{\delta^2 \varphi(x)}{\delta x^2} - \frac{\gamma_{40}}{4!} \cdot \frac{\delta^3 \varphi(x)}{\delta x^3}} \right] - m^2$$

In these formulae all the terms including derivatives of $\varphi(x)$ are dependent upon any anormality there may be in the distributions. If these are wholly normal, the calculations may be considerably simplified by neglecting these terms, except the one including the coefficient $\frac{\gamma_{22} + 2\gamma_{11}^2}{2!}$.

By the aid of the last two formulae and certain other rules of computation given below, it is possible to calculate the effect upon y of selection in x without having to resort to derivation and integration. The term $\int_{x_1}^{\infty} \varphi(x) dx$ is simply the usual integral of the normal curve, for the part of it outside the value x_1, and available in any table of this integral. It is only to be observed that x_1 in this case must be expressed in units of σ_x and in relation to the mean, so that if x_1 is taken as the absolute numerical value, the value to be used here is $(x - \bar{x})/\sigma_x$. The values of $\varphi(x)$ are also tabulated in several textbooks, e. g. by CHARLIER (1920) as »Tafel III». The value of $\varphi(x)$ for $x = (x - \bar{x})/\sigma_x$ should be ascertained. The derivatives of $\varphi(x)$ are also severally tabulated, the 3rd and 4th again by CHARLIER (1920) as »Tafel IV and V». If only a table of $\varphi(x)$ is available, the three first derivatives may be computed from the following formulae

$$\frac{\delta \varphi(x)}{\delta x} = -\frac{1}{\sigma_x} \cdot \frac{x - \bar{x}}{\sigma_x} \cdot \varphi(x)$$

$$\frac{\delta^2 \varphi(x)}{\delta x^2} = \frac{1}{\sigma_x{}^2} \cdot \left[\left(\frac{x - \bar{x}}{\sigma_x} \right)^2 - 1 \right] \cdot \varphi(x)$$

$$\frac{\delta^3 \varphi(x)}{\delta x^3} = -\frac{1}{\sigma_x{}^3} \cdot \left[\left(\frac{x - \bar{x}}{\sigma_x} \right)^3 - 3 \cdot \left(\frac{x - \bar{x}}{\sigma_x} \right) \right] \cdot \varphi(x)$$

$$\varphi(x) = \frac{1}{\sqrt{2\pi}} \cdot e^{-\frac{(x - \bar{x})^2}{2 \cdot \sigma_x{}^2}}$$

The formulae of m and $\sigma_{yr}{}^2$, finally, include certain coefficients, so-called standardized seminvariants, γ_{ij}, which are derived from the λ_{ij} coefficients of a later formula. The λ_{ij}, again, are functions of different moments

of x and y. The moments in this case are calculated from the mean, and may be defined as follows:

$$\nu_{10} = S\,[(x-\bar{x})]/_N$$
$$\nu_{01} = S\,[(y-\bar{y})]/_N$$
$$\nu_{20} = S\,[(x-\bar{x})^2]/_N$$
$$\nu_{02} = S[(y-\bar{y})^2]/_N$$
$$\nu_{11} = S[(x-x)(y-\bar{y})]/_N$$
$$\nu_{21} = S[(x-\bar{x})^2\cdot(y-\bar{y})]/_N$$
$$\nu_{30} = S[(x-\bar{x})^3]/_N$$
$$\nu_{13} = S[(x-\bar{x})(y-\bar{y})^3]/_N$$

and so on.

If the moments and seminvariants are calculated about the mean $\bar{x}$, $\bar{y}$, the coefficients λ_{ij} are obtained as follows:

$$\lambda_{10}=0 \qquad \lambda_{12}=\nu_{12} \qquad \lambda_{40}=\nu_{40}-3\cdot\nu_{20}^2$$
$$\lambda_{01}=0 \qquad \lambda_{30}=\nu_{30} \qquad \lambda_{04}=\nu_{04}-3\cdot\nu_{02}^2$$
$$\lambda_{20}=\nu_{20} \qquad \lambda_{03}=\nu_{03}$$
$$\lambda_{02}=\nu_{02} \qquad \lambda_{22}=\nu_{22}-\nu_{20}\cdot\nu_{02}-2\cdot\nu_{11}^2$$
$$\lambda_{11}=\nu_{11} \qquad \lambda_{31}=\nu_{31}-3\cdot\nu_{20}\cdot\nu_{11}$$
$$\lambda_{21}=\nu_{21} \qquad \lambda_{13}=\nu_{13}-3\cdot\nu_{02}\cdot\nu_{11}$$

Then, finally, each coefficient γ_{ij} is computed from the corresponding λ_{ij} by the formula:

$$\gamma_{ij}=\frac{\lambda_{ij}}{\sigma_x^{\,i}\cdot\sigma_y^{\,j}}$$

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ROBERT LAMM: A case of abnormal meiosis in *Lycopersicum esculentum.*

In the summer of 1941 Mr. E. NILSSON of the Plant Breeding Station, Gullåker, Hammenhög, Sweden, found a line of the tomato variety »Danish Export» consisting of 13 morphologically normal and fertile plants and 6 aberrant and sterile ones.

One of the normal plants was lost. From the remaining 12 normal plants seed was harvested, which was kindly presented for further investigations to Mr. B. HYLMÖ of the Horticultural Research Station at Alnarp. Four of these progenies entirely consisted of normal plants, eight of normal and aberrant plants in the ratio 127 : 50.

Young anthers of one of these aberrant plants were smeared by the author, fixed in diluted Navashin solution and stained in gentian violet. This plant proved to be trisomic. Diakinesis and first metaphase were normal. The odd chromosome either occurred as a univalent or together with its homologues formed a trivalent. At the end of the first anaphase the chromosomes had an hour-glass appearance. During first telophase they arranged themselves at each pole into a plate, whereupon their centromeres divided. At the end of this stage each telophase group thus contained the somatic chromosome number. The numbers of bodies of the interphase nuclei were also counted and proved to be unreduced. At second metaphase into each pollen mother cell two metaphase plates were formed consisting of already divided chromosomes. During the second anaphase these were distributed at random to the poles, and as a rule four nuclei were formed, though with rather irregular chromosome numbers. All the pollen grains were poor. Thus, the abnormality at meiosis consisted in the centromeres already dividing during first telophase instead of during the second metaphase.

Further investigations will perhaps show whether this abnormal form of meiosis is genotypically controlled and characteristic of all the aberrant individuals of these tomato lines.

ALBERT LEVAN and PER ARTUR OLSSON: On the decreased tendency to bolting in tetraploids of mangels and sugar beets.

A field trial for testing the bolting tendency of various root crops was sown this year (1943) at Svalöf. Owing to the extreme earliness of the spring, this year was especially suited for such a study. The field trial was sown on March 9th. Each variety to be tested was represented by a row ten m. in length, repeated three times in different parts of the field. The seed germinated after about one month. Thinning was carried out on May 17th. The first signs of bolting could be observed at the end of May.

In the present report only a few results from the artificially produced polyploids included in the experiment will be mentioned. There were three tetraploids together with their diploid controls, viz. Vilmorin and Hilleshög sugar beets and Barres mangels. The frequency of bolting was recorded on different occasions during the season. A count on September 7th is given in Table 1.

TABLE 1. *The number of bolters on September 7th, 1943.*

Repetition	Vilmorin						Hilleshög						Barres					
	2x			4x			2x			4x			2x			4x		
	Total	Bolters	% Bolters	Total	Bolters	% Bolters	Total	Bolters	% Bolters	Total	Bolters	% Bolters	Total	Bolters	% Bolters	Total	Bolters	% Bolters
a	45	33	73,3	45	15	33,3	45	19	42,2	46	26	56,5	50	41	82,0	34	3	8,8
b	39	29	74,4	49	13	26,5	42	20	47,6	46	20	43,5	50	32	64,0	38	4	10,5
c	50	43	86,0	49	16	32,7	49	32	65,3	48	18	37,5	44	34	77,3	47	6	12,7
Total	134	105	78,4	143	44	30,8	136	71	52,2	140	64	45,7	144	107	74,3	119	13	10,9

It was early noticed that there were very striking differences in the bolting tendency between diploids and tetraploids. As seen from the table, the tetraploids of all the three materials exhibited less tendency to bolting than the diploids. The greatest difference in this respect was found in the mangels, where 60—80 % of the diploids bolted, while only some 10 % of the tetraploids went to seed. A striking difference is also found in the Vilmorin sugar beets, while the Hilleshög variety behaved somewhat differently. The difference between the bolting of diploids and tetraploids in the Hilleshög variety was only a few per cents, and in one repetition the tetraploids even had more bolters than the diploids.

The decreased tendency to bolting in the tetraploids must be regarded as a favourable quality from a practical view-point. It is probably connected with the general tendency to a slower development and a later ripening often observed in artificial tetraploids and recently studied in tetraploid sugar beets and mangels by LEVAN (1943). The fact that the least significant difference in bolting between diploids and tetraploids is met with in the Hilleshög variety of sugar beets should be viewed in connection with the very slight tendency to bolting found in the diploid Hilleshög seed. If the chromosome doubling within sugar beets and mangels generally brings about a decreased tendency to bolting, this should be most easily traced within materials which in the diploid state may readily be brought to bolting. In such a material as Hilleshög, on the other hand, in which for years selection has been made against bolting under conditions favourable for the detecting of the bolting quality, a minimum tendency to bolting has probably been reached in the diploid, which perhaps can hardly be expected to be lowered in the tetraploid stage.

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ALBERT LEVAN: On the ubiquity of the camphor reaction of yeast.

Recent investigations by the present writer, made at the Central laboratory of the Stockholm Brewery Co., show that the so-called camphor reaction of yeast, first studied by BAUCH (1942) and by him thought to be something exclusive to camphor, is by no means limited only to camphor and some terpene derivatives. Its general occurrence can be demonstrated, however, only by means of quantitative tests, i. e. the yeast cells must be treated with exact concentrations of the different substances.

In the present study about 50 organic substances were tested, including 19 aliphatic, 11 alicyclic, 16 aromatic and 2 heterocyclic compounds. Most of the substances were such as are known with certainty to have narcotic effects on animals. Considering the causal conditions of the narcotic activity, as far as these are known, it may be rather safely concluded that the other substances tested also have more or less narcotic properties. It was found that most of the substances were able to induce the camphor reaction. Thus, the reaction was demonstrated in 18 aliphatic, 6 alicyclic, 14 aromatic and 2 heterocyclic substances.

The camphor reaction varied a great deal in type. It was found that the significant feature of the reaction was not so much the change in cell shape as *the hanging together in colonies of the cells.* This could be directly referred to the deviating course of the budding process observed to take place under influence of the substances. In some substances (e. g. veronal, sulphonal, adaline, cyclohexane, phenylethyl alcohol, toluene, thiophene) the reaction was incomplete, normal cells occurring intermingled with camphor forms even in the strongest concentrations allowing cellular growth. In the aliphatic and alicyclic substances the camphor reaction was often similar in type, the cells of the colonies growing out into long tube-like or irregularly swollen formations several times the volume of normal yeast cells. Especially extreme cases were ether, acetone, urethane, tertiary butyl alcohol and, of course, camphor. The aromatic monocyclic substances brought about less extreme changes, the cells of the camphor colonies usually being rounded, often with a somewhat undulated outline. The few bi- and poly-cyclic aromatic substances earlier tested by LEVAN and SANDWALL (1943) had no perceptible camphor effect, with the possible exception of naphthalene acetic acid, which showed certain tendencies to inducing the camphor reaction.

The activity threshold of the camphor reaction was found to show the same positive correlation to the water solubility of the substances as was earlier observed to be valid in the c-mitotic reactions by LEVAN and ÖSTERGREN (1943) and ÖSTERGREN and LEVAN (1943). For the present purpose one instance of this condition may suffice: The solubility (roughly estimated in wort) and the activity of the studied aliphatic alcohols and one halogenated alcohol were as follows:

	Solubility in mol	Activity in mol
Methyl alcohol	∞	1—(0,5)
Ethyl alcohol	∞	1—0,5—(0,2)
Tertiary butyl alcohol	∞	(0,5)—0,2—(0,1)

	Solubility in mol	Activity in mol
Amylene hydrate	2—1	0,2—(0,1)
Normal primary butyl alcohol	0,1—0,05	0,1—(0,05)
Chlorbutol	0,08—0,04	0,02
Octyl alcohol	0,01—0,005	0,01—(0,005)

The main trend of the solubility-activity correlation shown in this case was almost persistently repeated within all groups of related substances studied in the present investigation.

As discussed in some detail by ÖSTERGREN (1944), the c-mitosis, the c-tumour reaction, the camphor reaction, and the narcotic reactions of animals form a group of closely related phenomena. The following features are common to all these reactions: (1) the disconnecting of various vital functions, usually superior regulating functions, such as *the growth control* of the yeast cells in the case of the camphor reaction; (2) the reversibility of the reactions; (3) the fact that they all seem to obey the same solubility-activity correlation; (4) the fact that the ability of the substances to induce the reaction is largely independent of chemical structure and is probably more or less strongly present in most groups of organic substances.

The thresholds of the different reactions may coincide within the same material, but each reaction may also show a great measure of specificity and individuality. Poison action and lethality also seem to be connected with the narcotic action, and have been found to follow, broadly speaking, the same solubility correlation. In the opinion of ÖSTERGREN and myself they may be conceived as a narcosis of such vital processes as cannot be temporarily disconnected without serious damage to the organism. The most probable reason of the failure to demonstrate the camphor activity in some of the tested substances is that in these cases the activity is concealed by lethality and poison effect. This assumption is favoured by the fact that the poison threshold in some cases lies far above the camphor reaction threshold and in other cases very close to it. It seems, then, only natural to assume that the poison threshold in some cases should be situated at a lower concentration than the threshold of the camphor reaction.

A complete account of the yeast experiments will be published shortly.

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PETER BERNSTRÖM: Two new hybrids in *Lamium*.

In 1941 the production of tetraploid *Lamium purpureum* L. and *L. amplexicaule* L. was reported as well as the attempt to attain a synthesis of *L. hybridum* VILL. and *L. intermedium* FR. with their help (BERNSTRÖM, 1941). This has not yet been successful, but during the work two new species hybrids were obtained which seem to be worth describing.

As stated in 1941, *purpureum* and *amplexicaule* are diploid, their chromosome number being n = 9, while *hybridum* and *intermedium* are tetraploid, and the only hybrid that can be produced between these four species is *amplexicaule* × *hybridum*, which is thus a triploid and quite sterile (MÜNTZING, 1926; JÖRGENSEN, 1927). By substituting tetraploid *purpureum* and *amplexicaule* for the diploids in all cross combinations, in which the latter fail to set seed, it has now been possible to produce two new species hybrids, viz. *hybridum* × tetraploid *purpureum* and *intermedium* × tetraploid *amplexicaule*. A table will make the cross results clear. In order to give the greatest possible evidence in the table the last crossings are also included in spite of the fact that there are only seeds to be accounted for, but the crossings are made with such care that the seeds are certainly genuine, although they will probably not all germinate.

TABLE 1. *Results from crossing trials in 1941—1943.*

Cross	Flowers pollinated		Hybrids obtained	Seeds obtained
Purpureum (n = 9) × *amplexicaule* (n = 18) ..	21		—	
Reciprocal cross	11		—	
Purpureum (n = 18) × *amplexicaule* (n = 9) ..	21		—	
Reciprocal cross	23		—	
Purpureum (n = 18) × *amplexicaule* (n = 18) ..	145	(14) [1]	—	
Reciprocal cross	76	(11)	—	
Purpureum (n = 18) × *hybridum* (n = 18)	103	(1)	—	
Reciprocal cross	51		20	18
Purpureum (n = 18) × *intermedium* (n = 18) ..	80		—	
Reciprocal cross	202	(21)	—	
Amplexicaule (n = 9) × *hybridum* (n = 18)	23		3	
Reciprocal cross	38		43	52
Amplexicaule (n = 18) × *hybridum* (n = 18) ..	123	(10)	—	
Reciprocal cross	126	(11)	—	
Amplexicaule (n = 18) × *intermedium* (n = 18)	63	(7)	25	51
Reciprocal cross	124		16	34

For the cross experiments there have been used 2 diploid and 2 tetraploid lines of *purpureum*, 2 diploid and 4 tetraploid lines of *amplexicaule*, 5 lines of *hybridum*, and 2 lines of *intermedium*. One *hybridum* line is of Swiss origin, but with the exception of this the material is Swedish. Every possible

[1] The figures within brackets indicate the number of flowers, included in the totals, in which the styles were half-cut in order to investigate whether the crossing ability was thereby facilitated. Flowers treated in this way never set seed.

combination has not been tried between the different lines, nor have the attempts of MÜNTZING and JÖRGENSEN to cross the original species been repeated by me as yet, except for the only successful combination.

As will be seen from the table, the doubling of the chromosome number of the two diploid species has not been able to bring forth a hybrid between them, either both species are tetraploid or one is tetraploid and the other diploid. However, the second combination in its four directions has been tried to such a small extent as not to permit any conclusions. From the table it will be seen as well that the new species hybrids are rather easily obtained, though the mother species is of great or even decisive importance in this connection. The one-sidedness in the cross between *hybridum* × tetraploid *purpureum* may be connected with the dwarfishness of this hybrid, about which more will be said further on. In trying to get the reciprocal cross, 2 *purpureum* lines were combined with 5 *hybridum* lines in 8 different ways.

It is interesting to note that the only hybrid previously obtained, viz. diploid *amplexicaule* × *hybridum*, cannot be produced if *amplexicaule* is tetraploid. 2 out of the 3 line crosses which gave 1,24 hybrids per flower did not give a single hybrid plant or seed when the diploid *amplexicaule* was replaced by the tetraploid one, nor did 4 new ones. Thus it will not be possible to test the hypothesis of getting plants transgressing to *intermedium* from the progeny of such a hybrid, at least with the material now available (cf. MÜNTZING, 1926).

Finally, it is remarkable that through the new hybrids it has been possible to unite all 4 *Lamium* species into a chain, in which *hybridum* and *amplexicaule* are the connecting links; however, these are not equivalent chromosomally.

The new hybrids were found to be different both in morphology and fertility. 20 plants of *hybridum* × tetraploid *purpureum* were obtained from 3 line combinations, including 2 lines from each species. In spite of this, they have all been dwarfish, though to a varying degree, the main shoot of the highest plant being 6,3 cm. and that of the lowest one 3,3 cm. Both these plants were from the same line combination, though raised in different generations. The height of the parents was something like 40 cm. The morphology of the hybrid plants varied in other respects, too. Thus, some of the plants had only rudimentary branches, in which case they were often low and sometimes large-leaved, others had plenty of branches, which were often longer than the main shoot; this never occurs in the parents. The shape and size of the leaves (the greatest length measured was 22 mm.) as well as their indentation and the form of their dents varied to some extent even in the same plant. There was a similar variation in the size and morphology of the calyces. However, judging from the largest ones, the calyces were the organs which had diminished in size least in comparison with those of the parents. This observation was made only by eye, because accurate measurement would have necessitated removal of the calyces from the plant, and this was avoided in order not to spoil any possibilities for seed-setting. (Some of the plants were used for microscopical purposes.) Of all the organs, the flowers were the most uniform and equal in size. In the first generation in 1943 (June—July) the flowers were open and about 8 mm. in length, while the flowers

of the second generation (Sept.—Oct.) were all closed and only about 5 mm. in length. The flowers of the parents are about 19 mm. in *purpureum* and about 14—15 mm. in *hybridum*.

The variation outlined above could not be caused by genotypical differences, both parent species being autogamous and uniform. Nor is it likely to have been due to varying chromosome numbers, although, as yet, only two plants have been examined, both having $2n = 36$. Most likely, the variation between the plants was caused by a low vitality and a discordant gene constitution, also appearing in a delay in the starting of flowering of about 11 days, as compared with that of the parents.

The female fertility of the hybrids was probably nil, as no seed has as yet been obtained, perhaps partly because of the pollinative difficulties which were present. The pollen fertility ranged between 1 and 8 %, calculated from the number of filled pollen grains in aceto-carmine slides. Nevertheless, no guarantee can be obtained for the function of a grain only from its mere appearance (MÜNTZING, 1929). Similarly, the male fertility in tetraploid *purpureum* ranges from 78 to 98 % and in *hybridum* from 89 to 99 %; it differs in different lines. The female fertility in the tetraploid *purpureum* ranges from about 30 to 60 % and in *hybridum* from 80 to 98 %.

In contradistinction from the hybrids described above, the hybrids tetraploid *amplexicaule* × *intermedium* and *vice versa* were vegetatively well developed and partially fertile. They were obtained from the crossing of 3 different lines of *amplexicaule* with the same *intermedium* line, and were almost as uniform in appearance as the parents, which are autogamous. Externally the hybrids most resembled *intermedium*; they were only about 50 cm. in height, however, whereas *intermedium* was about 60 cm. and *amplexicaule* about half of that. They had a somewhat more extended inflorescence, too (most distinct in the beginning of the flowering), more deeply lobated leaves, a greater number of small branches, a greater variability in the size of the flowers, etc., all of this being an inheritance from *amplexicaule*. *Intermedium* has chasmogamous flowers only, about 21 cm. in length; *amplexicaule* has chasmogamous as well as cleistogamous flowers, the former being 21—27 mm., rarely shorter, and the latter 4—5 mm. The hybrids, too, showed both kinds of flowers, and, in addition, a series of transition forms; the flowers were 5—24 mm. in length. The flowering of the hybrids started between that of the parents, closer to that of *intermedium* than of *amplexicaule*, which is the earlier one.

Two hybrid plants differed morphologically from the other, one by a more abundant ramification and a darker colour of the branches, and the other by a slower growth, a darker colour of the leaves, and exclusively cleistogamous flowers. They were not sister plants. Their chromosome numbers have not yet been examined. Some of the hybrids were withdrawn from morphological analysis, all their branches being cut for bulk fixation.

The seed-setting of the hybrids was very low. Of 26 plants, which germinated rapidly and therefore had time to complete most of their flowering before the cold of the autumn put a stop to it, and which were not truncated too much at the bulk fixation, 18 plants set 1—2 seeds each, rarely any more. Altogether 29 seeds were harvested. As in the hybrids *hybridum* × tetraploid

purpureum the co-operation between the anthers and the style in regard to autogamy often failed, both in the chasmogamous and in the cleistogamous flowers, and because of this the female fertility was lowered, probably to one-half. In tetraploid *amplexicaule* this fertility varies between about 20 and 60 %, and in *intermedium* from about 65 to 85 %. In the hybrids, the percentage of filled pollen grains was found to be 12—32 %, in tetraploid *amplexicaule* 76—96 %, and in *intermedium* 92—99 %. Before the low female fertility in the hybrids had been discovered, 53 flowers were pollinated with tetraploid *purpureum*, without result.

Finally, if any reader of the above should be in a position to send to the author some seeds of *Lamium garganicum*, *Aleppicum*, *Ehrenbergii*, *macrodon*, *Mauritanicum*, *moschatum*, *ponticum*, or any other annual *Lamium* species or variety that is not indigenous to northern Europe, he would be very much indebted.

Appendix. — Since this paper was written, a grain was obtained from a *hybridum* × tetraploid *purpureum* plant. This was put in a greenhouse at the end of October together with several sister plants, as they were still vegetating. The grain is well-developed but rather small.

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